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Gestion du carbone et de l'azote et développement végétatif des arbres forestiers en relation avec les variations environnementales

Pascale Maillard

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UMR 1137 Ecologie et Ecophysiologie Forestières
Equipe Fonctionnement intégré de l'arbre et de l'écosystème
IFR 110 Ecosystèmes Forestiers, Agroressources, Bioprocédés et Alimentation
Ecole doctorale RP2E

Gestion du carbone et de l'azote et développement végétatif des arbres forestiers en relation avec les variations environnementales

Habilitation à Diriger des Recherches

Soutenance le 31 mai 2012 à 14h00

Devant la commission d'examen

Rapporteur :	**Th. Améglio**	**DR, INRA Clermont-Ferrand**
	J. Ghashghaie	**Pr, Université Paris Sud**
	M.P. Prud'homme	**Pr, Université de Caen**
Examinateur :	**P. Dizengremel**	**Pr, Université de Lorraine et Président du Jury**
	C. Damesin	**Pr, Université Paris Sud**

Présentée par Pascale MAILLARD
Docteur en Physiologie végétale
Chargée de Recherches INRA

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Remerciements

Je tiens à remercier chaleureusement les membres du Jury, pour m'avoir fait l'honneur de bien vouloir examiner ma demande d'habilitation à diriger les recherches : Mesdames et Messieurs les Professeurs Jaleh Ghasghaie, Marie-Pascale Prud'homme, Claire Damesin, Pierre Dizengremel, et Thierry Améglio Directeur de Recherches à l'INRA.

Mes remerciements vont en particulier au Professeur Pierre Dizengremel qui m'a fait l'honneur d'accepter d'être mon parrain scientifique.

Je remercie tous mes collègues de travail de l'UMR EEF, et en particulier celles et ceux avec lesquels j'ai le plus travaillé ces dernières années : Dominique Gérant, Nathalie Bréda, Daniel Epron, André Granier, Claude Bréchet, Bernard Clerc, Pascal Courtois, François Gérémia, Jean-Marie Gioria, Patrick Gross, Christian Hossann, et Jacqueline Marchand.

Je remercie enfin tous les étudiants cités ici dont le travail a grandement contribué à l'avancement des travaux de recherche présentés.

Je dédie ce manuscript à Eliane Deléens, à ma chère Maman et à mes enfants Emilie et Vincent.

To my best friend Deborah

FICHE SIGNALETIQUE :

Demande de soutenance

de l'Habilitation à diriger des Recherches de l'Université de Lorraine

Arrêté du 23 novembre 1988 relatif à l'Habilitation à diriger des Recherches

Formulaire à compléter et à retourner ***8 semaines minimum avant la date de soutenance*** *par voie papier à : Direction de la Recherche et de la Valorisation (DRV)* – UNIVERSITE DE LORRAINE 34, cours Léopold- CS 25233 – 54052 Nancy Cedex

I - INFORMATIONS CONCERNANT LE CANDIDAT

NOM - PRÉNOM ~~M.,~~ Mme, ~~Mlle~~ (*) MAILLARD PASCALE.....
Université de rattachement :…Université de Lorraine
……
Parrain scientifique : Professeur Pierre Dizengremel, Professeur des Universités

DIPLOME DE DOCTORAT en (discipline) Physiologie Végétale.....

Université Pierre et Marie Curie (Paris VI) ..... Année 1987.....

II - INFORMATIONS CONCERNANT L'HDR

Ecole Doctorale Ressources Procédés Produits Environnement…RP2E n°410….....

DISCIPLINE Sciences agronomiques et forestières, biologie et écologie, biotechnologies .....

Section CNU /EPST .....

Thème de la recherche : Gestion du carbone et de l'azote et développement végétatif des arbres forestiers en relation avec les variations environnementales .....

.....

.....

.....

DATE définitive de la soutenance :

Jeudi 31 mai 2012..... **à** 14..... **h** .....

Lieu : Faculté des Sciences de Nancy, Amphithéatre 7.....

Les éventuels compléments d'informations sont à communiquer le plus rapidement possible au SRED

III – INFORMATIONS CONCERNANT LES RAPPORTEURS ET LE JURY

NOM ET QUALITÉ DES 3 RAPPORTEURS :

(A choisir au sein de la liste validée par l'école doctorale (fiche signalétique demande d'inscription à l'HDR)

Indiquer qualité, adresse professionnelle, numéro de téléphone et de télécopie, e-mail, section CNU et Ecole Doctorale

~~M.~~, Mme, ~~Mlle~~ (*) GHASGHAIE JALEH Professeure des universités, Département d'Ecophysiologie Végétale, Laboratoire d'Ecologie, Systématique et Evolution (ESE), CNRS-UMR8079, Bâtiment 362, Université Paris-Sud (XI), F-91405-Orsay, Tel: ++33(0)1.69.15.63.59. Fax: ++33(0)1.69.15.72.38. E-mail: jaleh.ghashghaie@u-psud.fr.....

.....

~~M.~~, Mme, ~~Mlle~~ (*) PRUD'HOMME MARIE-PASCALE Professeure des universités, UMR A 950 - Laboratoire d'Ecophysiologie Végétale, Agronomie et nutrition N.C.S., Université de Caen Esplanade de la Paix, 14032 Caen cedex. Tél : ++33(0)2.31.56.55.24. E-mail: marie-pascale.prudhomme@unicaen.fr.....

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M., ~~Mme, Mlle~~ (*) AMEGLIO THIERRY, Directeur de Recherches, UMR INRA-547 PIAF, INRA- Université Blaise-Pascal U.F.R. S. & T. - P.I.A.F.24, Avenue des LANDAIS 63177 AUBIERE Cedex. Tél ++33 (0)4.73.62.43.69. Fax ++33 (0)4.73.62.44.54; école doctorale des Sciences de la Vie et de la Santé de Clermont Ferrand. E-mail Thierry.Ameglio@clermont.inra.fr.....

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NOM ET QUALITÉ DES AUTRES MEMBRES DU JURY :

EXAMINATEURS :

Indiquer qualité, adresse professionnelle, numéro de téléphone et de télécopie, e-mail, section CNU et Ecole Doctorale

~~M.,~~ Mme, ~~Mlle~~ (*) DAMESIN CLAIRE, Professeure des universités, Université Paris-Sud XI, UMR 8079, Bât. -362, 15, rue Georges Clemenceau, 91405 Orsay cedex. Tél

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.....

.....

M., Mme, Mlle (*) .....

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M., Mme, Mlle (*) .....

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M., Mme, Mlle (*) .....

.....

<u>INVITES EVENTUELS</u> (ne participent pas à la délibération du jury) :

M., Mme, Mlle (*) .....

.....

M., Mme, Mlle (*) .....

.....

(*) Rayer les mentions inutiles

<u>IV. VALIDATION DE LA PROPOSITION DEFINITIVE DE JURY ET DE RAPPORTEURS PAR L'ECOLE DOCTORALE :</u>

Nom, Date et signature :

A remplir par la DRV
Date de dépôt du dossier :

Copie du diplôme de doctorat

R É P U B L I Q U E F R A N Ç A I S E

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE

UNIVERSITÉ PARIS VI

DIPLÔME DE DOCTEUR

Vu l'arrêté du 30 mars 1992 relatif aux études doctorales ;

Vu les titres initiaux produits par Mlle Pascale MAILLARD, née le 26 Mai 1960 à SURESNES (HAUTS DE SEINE) ;

Vu les pièces constatant que l'intéressée a présenté en soutenance, conformément aux règlements, à la date du 15 Décembre 1987, une thèse ou un ensemble de travaux portant sur le sujet : ETUDE DU DEVELOPPEMENT VEGETATIF DU TERMINALIA SUPERBA ENGLER ET DIELS EN CONDITIONS CONTROLEES : MISE EN EVIDENCE DE RYTHMES DE CROISSANCE.;

devant un jury constitué au sein de l'Université Paris VI présidé par MR. MIGINIAC et composé de MR CORBASSON,Mme JACQUES,MR JACQUES,MR MIGINIAC,MR MILLET,MR MAUGET;

Vu la décision dudit jury prononçant l'admission de l'intéressée ;

le DIPLÔME DE DOCTEUR de l'Université Paris VI, spécialité : .

est conféré à **Mlle Pascale MAILLARD**, avec la mention très honorable,

pour en jouir avec les droits et prérogatives qui y sont attachés.

Fait à Paris, le 16 Mars 2011

Le titulaire

Le Président

JEAN-CHARLES POMEROL

Le Recteur d'Académie
Chancelier des universités

PATRICK GERARD

N° PARVI 8170371 201100914

Curriculum vitae

ETAT CIVIL

Pascale MAILLARD née le 26 mai 1960 à Suresnes (92)
Célibataire – 2 enfants
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Adresse professionnelle : UMR INRA-UHP 1137, IFR 110, Ecologie et Ecophysiologie Forestières, INRA, Forêt d'Amance, 54280 Champenoux, France
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DIPLOMES

1987. Doctorat de Physiologie végétale, direction Pr Emile Miginiac, Université Paris VI, mention très honorable avec les félicitations du jury.
1984. DEA de Physiologie Végétale, direction Dr. Antoine Trémolières, Université Paris VI, mention bien.
1983. Maîtrise de Biologie des organismes et des populations, mention Physiologie Végétale, Université Paris VI.
1982. Licence de Biologie des organismes et des populations, option Université Paris VI.
1981. DEUG, Science de la Nature et de la Vie, Université Paris XII, mention assez bien.
1**978**. Baccalauréat D, Académie de Créteil.

ACTIVITES PROFESSIONNELLES

2002 à ce jour. Chargée de recherches 1ère classe, UMR EEF, Equipe Arbeco, INRA Nancy (54280).
2002. Mutation au Département Ecologie des forêts, prairies et milieux aquatiques (EFPA).
1995. Détachement pour 4 ans à l'Unité d'Ecophysiologie Forestière, INRA Nancy (54280).
1992. Chargée de recherches 1ère classe, Département Environnement et Agronomie, UMR PIAF, Clermont-Ferrand (63000).
1989. Chargée de recherches 2ème classe titulaire.
Fin 1988. Mutation à l'INRA, laboratoire de Bioclimatologie, Clermont-Ferrand (63000).
1988. Stage d'un an sur le métabolisme des glucides et la modélisation des flux de ^{14}C chez *Zea mays* au laboratoire de métabolisme et nutrition des plantes (Direction : Pr. Jean-Louis Prioul et Jean-Paul Rocher), Université d'Orsay (91405).
1988. Détachement à l'INAPG, au laboratoire de Bioclimatologie pour 1 an.
1987. Chargée de recherches 2ème classe stagiaire, Département Bioclimatologie. Détachée au CNRS, Phytotron, Gif-sur-Yvette (91272).

RESPONSABILITES PEDAGOGIQUES ET EXPERTISES SCIENTIFIQUES

Participation à l'encadrement de post-doctorants : 2.
Participation à l'encadrement de thèses : 11.
Encadrant de DEA ou Masters : 16.
Encadrant niveau ingénieur : 2.
Encadrant de BTS, DUT, IUT : 4.
Encadrant de scolaires : 2.
Rapporteur soutenances Master FAGE (M1, M2) depuis 2007.
Membre de comités de thèse : 9.
Examinateur dans jury de thèse : 2.
Membre élu du conseil scientifique UMR INRA-UHP 1137 Nancy en 2003.
Membre élu du conseil scientifique du département Environnement et Agronomie de 2002 à 2006.
Evaluateur de projets innovants INRA des départements EA et EFPA.
Représentant du Conseil Scientifique et expert nommé par le département EA pour l'évaluation d'UMRs INRA-Université (Ephyse, 2003 ; Oenologie-Ampélologie à Bordeaux, 2006 ; Unité PSH à Avignon, 2007).
Représentant du personnel élu en 2011 à la Commission Administrative Paritaire Nationale (CAPN) chargés de recherche de l'INRA.

LIAISONS EXTERNES A L'INRA

Membre des commissions de spécialistes CSE 64/68, 67/68 et 66/69 à l'Université Nancy I, et évaluateur de dossiers de recrutement de MCF et d'ATER de 2002 à 2009 inclus.

Membre élu du Conseil de la SFPV affiliée à la FESPP jusqu'en 2008.
Membre de sociétés savantes (SVPV, FESPP, GERB, GEA, SFIS).
Membre de réseaux (eCOST Sibae, Gip Ecofor, Netcarb, …)
Animation occasionnelle Sciences en fêtes Université Nancy 1.

PARTICIPATION A DES CONTRATS DE RECHERCHE

- ***Programmes régionaux***

2001-2003. Projet après tempête Forbois Région Lorraine (Coordinateurs: J. Garbaye, P. Maillard et M.B. Triboulot-Bogeat ; 25 k€).
2006-2007. Projet IFR IFR 110 "Génomique, Ecophysiologie et Ecologie Fonctionnelle" Nancy (Coordinateur: P. Maillard ; 5 k€).
2007-2010. Projet émergent Région Lorraine «Dynamiques saisonnières des composés azotés de réserve chez le chêne sessile et le hêtre en forêt lorraine. Mise au point d'un outil de diagnostic simple de vitalité des arbres » (Coordinateur: P. Maillard ; 10 k€).
2008-2011. Projet émergent Région Lorraine Mise en place d'un Taillis à Courte Rotation (TCR) atelier en Lorraine : Comment optimiser et maintenir durablement la production de biomasse ligneuse à fins bioénergétiques ? (Coordinateur : N. Marron ; 10 k€).
2008-2013. Projet Gluciflor, Région Champagne (Coordinateur : N. Vaillant).

- ***Programmes nationaux***

2000-2006. Projet CPER/Doc UP « Caractérisation écophysiologique d'espèces forestières majeures pour leur maîtrise sylvicole » (Coordinateur : D. Bonal, INRA, Kourou, Guyane).
2008-2009. Projet innovant Dpt. EFPA. « Le chêne est-il mycohétérotrophe au printemps ? » (Coordinateurs : P. Maillard et J. Garbaye ; 9 k€).
2010-2011. Projet innovant Dpt. EFPA Suivi à haute résolution (spatiale et temporaire) de l'allocation du carbone et de l'azote à l'échelle arbre - sol d'une hêtraie lorraine par triple marquage isotopique (13C, 15N, 2H) (Coordinateur : P. Maillard; 10 k€).
2008-2011. Projet ANR CATS "Integrated monitoring of carbon allocation in tree and soil" (Coordinateur : D. Epron: 182 k€).
2011-2014. Projet ANR systerra, Intens&Fix (Coordinateur : J.P. Bouillet).
Soumis début 2012. Projet ANR Fluxomix (Coordinateur Ph. Thaler).
2012. Labex ARBRE (Centre INRA Nancy (Coordinateur : Francis Martin).

- ***Programmes européens***

2000. Netcarb (net terrestrial carbon, projet européen) et réseau d'isotopistes (http://web2.wzw.tum.de/netcarb/).
2007: CarboEurope (Coordinateur INRA Nancy : A. Granier) (http://www.carboeurope.org/).
2008. Ecost Sibae.

- ***Coopération internationale***

Depuis 2010. Bilateral cooperation project between FRANCE (INRA) – JAPAN (JSPS) on the use of stable carbon isotope to understand the carbon cycle in the forest.
Soumis fin 2011. Projet finlandais sur 4 ans : "Ice encasement - a new winter stress for tree seedlings in the boreal forest?" (Coordinateur : Minna TURUNEN).

AUTRES ACTIVITES

Coordination de trois colloques nationaux en 1993 et 1998 (Paris) et 2009 (Caen).
Editeur de deux ouvrages collectifs INRA série Colloques.
Lecteur arbitre dans des revues internationales (*Plant Biology, Tree Physiology, Annals of Botany, Annals of Forest Sciences, Plant and Soil, Oecologia, Aquatic Ecology*).
Membre éditeur d'*ISRN Botany journal*.
Sauveteur secouriste du travail.

DISTINCTIONS

Thèse de doctorat, médaille de vermeil décernée par l'Académie d'Agriculture, France le 07 décembre 1988 (Rapporteur : Roger Jacques, Directeur du Phytotron, CNRS, Gif-sur-Yvette 91).

Lettre de recommandation et d'engagement du parrain

Nancy-Université
Université Henri Poincaré

ECOLOGIE ET ECOPHYSIOLOGIE FORESTIERES
UMR 1137 INRA-UHP

INRA
Institut National de la Recherche Agronomique

LETTRE de PARRAINAGE
pour la candidature de
Madame Pascale MAILLARD à une HDR

Madame Pascale MAILLARD, âgée de 51 ans, a obtenu le Doctorat en Physiologie végétale à l'Université Paris 6 en 1987 avec la mention «Très honorable et félicitations du jury». Elle a été recrutée la même année chargée de recherches 2ème classe INRA au Département de Bioclimatologie de Gif sur Yvette. En 1988, Mme Maillard obtient un détachement à l'INAPG (Institut National Agronomique Paris-Grignon) et effectue un stage de formation sur le métabolisme des glucides au laboratoire de métabolisme et nutrition des plantes de l'Université d'Orsay, ce qui lui permet d'acquérir dans ce domaine des compétences qu'elle fera fructifier dans la suite de sa carrière. A la fin de cette même année 1988, Mme Maillard obtient sa mutation à l'INRA de Clermont-Ferrand (département environnement et agronomie), toujours comme chargée de recherches. Titularisée en 1989, elle devient CR INRA 1ère classe en 1992. Après un détachement au laboratoire d'écophysiologie forestière INRA de Nancy à partir de 1995, elle y est définitivement mutée en 2002, le laboratoire étant devenu entre temps UMR 1137 EEF INRA/Nancy Université.

Au cours de sa thèse, Mme Maillard, entourée des grands spécialistes de l'époque, à Gif sur Yvette et Paris 6, a travaillé sur la floraison et, plus particulièrement, sur les effets de la lumière, des phytochromes et des hormones sur les mécanismes conduisant à cette floraison. Mme Maillard a aussi abordé, au cours de cette thèse, les mécanismes de la croissance rythmique endogène. Enfin, elle s'est familiarisée avec les techniques histologiques et cytologiques. Ce travail de doctorat, qui lui a valu la médaille de vermeil de l'Académie d'Agriculture (1988), a donné lieu à 4 articles publiés dans de bonnes revues. Le détachement obtenu la même année, dans un riche environnement scientifique à Orsay, lui permet d'abonder considérablement sa panoplie de techniques modernes d'étude des flux de carbone depuis l'atmosphère et entre compartiments des plantes (isotopie, HPLC, chromatographie phase gazeuse, enzymologie, modélisation,..). Les compétences acquises dans l'utilisation des isotopes stables va lui permettre d'initier son programme de recherche sur les flux de carbone et azote chez les arbres fruitiers lors de sa nomination à l'UMR PIAF de Clermont-Ferrand fin 1988. Au cours de cette période allant de 1989 à 1995, Mme Maillard a commencé à co-encadrer des étudiants et a aussi co-organisé deux colloques.

En 1995, Mme Maillard rejoint le laboratoire d'écophysiologie forestière de l'INRA Nancy, devenu depuis UMR 1137 EEF INRA/Nancy Université, et y exerce toujours actuellement ses activités. C'est à cette époque que j'ai pu plus particulièrement suivre ses activités, étant directeur du laboratoire universitaire de biologie forestière associé INRA puis directeur adjoint de l'UMR. Mme Maillard a étroitement collaboré avec ses collègues universitaires sur les impacts des stress abiotiques sur les arbres forestiers (chêne, hêtre, peuplier) tout d'abord principalement sur le métabolisme carboné, en apportant ses

compétences isotopiques et en installant à l'INRA les équipements nécessaires pour les mesures physiologiques et biochimiques. Plus récemment, au sein d'un groupe de travail sur la gestion du carbone et de l'azote chez des arbres adultes en situation naturelle, avec un intérêt particulier pour l'évolution des réserves au cours des saisons (en particulier protéines), Mme Maillard occupe une place clé de physiologiste/écophysiologiste entre les biochimistes/biologistes moléculaires et les écologistes. Je peux témoigner que l'apport de Mme Maillard à cette thématique, qui transcende les disciplines et permet les changements d'échelle, est essentiel.

Au niveau publications, Madame Maillard en comptabilise 23 dans des revues internationales à comité de lecture dont plus de 15 après sa thèse. Elle a participé à 9 chapitres d'ouvrages et 2 ouvrages. Elle fait état aussi d'une soixantaine de communications orales et posters dans des congrès. Madame Maillard a aussi effectué et effectue régulièrement des activités d'enseignement (entre 6 et 10h eq TD par an) en DEA/master à l'UHP Nancy 1. Madame Maillard a co-encadré une dizaine de thèses et plusieurs étudiants en DEA et master.

Madame Maillard a participé et participe actuellement à de nombreux programmes de recherche ainsi qu'à la coordination pour certain d'entre eux et possède aussi un très bon réseau de relations internationales.

Madame Maillard, en possession de techniques clés au service d'une thématique de recherche partagée en étroite collaboration avec des chercheurs et enseignants-chercheurs de l'UMR, démontre sa capacité à intégrer ses compétences au sein d'un laboratoire. Les qualités scientifiques de Mme Maillard la conduisant à une très bonne production d'articles, ses qualités humaines, sa participation à l'encadrement de jeunes et son implication dans la vie scientifique de l'UMR, me conduisent à **soutenir extrêmement favorablement la candidature de Madame Maillard à l'obtention de l'Habilitation à diriger des recherches**.

Nancy, le 8 Juin 2011

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Préambule

Mes premiers pas dans le monde de la recherche ont été effectués dans le laboratoire de Physiologie des Organes Végétaux après Récolte dirigé par le Professeur Daniel Côme et sous la férule du Docteur Françoise Corbineau (CNRS de Meudon, France). J'y ai expérimenté, dans le cadre d'un stage de maîtrise optionnel, l'impact d'un facteur de l'environnement (la température) sur la levée de dormance des graines de différentes espèces ornementales. Au cours de mon stage de DEA [124] réalisé au Phytotron (CNRS, Gif-sur-Yvette) j'ai ensuite découvert le monde des isotopes et du métabolisme des lipides grâce aux Docteurs Antoine Trémolières et Alain Lecharny (CNRS, Gif-sur-Yvette, France). J'y ai compris l'intérêt d'un marqueur radioactif, le carbone 14 (^{14}C), pour suivre l'incorporation des acides gras dans les membranes en croissance de la tige d'une chénopodiacée (*Chenopodium rubrum*), dont l'une des caractéristiques est d'exprimer une croissance de rythme circadien. Etant reçue dans les trois premiers de ma promotion, j'ai pu alors bénéficier d'une bourse du ministère (MIR) sur un sujet de thèse fléché qui m'intéressait beaucoup et qui concernait l'étude de la floraison et plus généralement du développement d'un arbre forestier tropical (nom vernaculaire Fraké ou Limba, *Terminalia superba*, famille des Combrétacées) reconnu pour la qualité de son bois, à la fois au Congo et en Côte d'Ivoire. Ce sujet était commandité par le CIRAD et plus particulièrement par un de ses départements, le Centre Technique Forestier Tropical de Nogent sur Marne, représenté dans le cadre de ma thèse par monsieur Michel Corbasson. Ma thèse a été dirigée par le Professeur Emile Miginiac, spécialiste en hormonologie végétale, et par Madame Monique Jacques Maître de Conférences à l'Université Pierre et Marie Curie (Physiologie Cellulaire et Moléculaire des Plantes, Paris VI). J'ai été accueillie puis suivie avec attention et bienveillance par Roger Jacques alors Directeur du Phytotron (CNRS, Gif-surYvette) et spécialiste de la lumière, des phytochromes, et de ses effets sur les plantes. J'ai approfondi mes connaissances sur l'influence du milieu, en particulier la température et l'éclairement (photopériode, qualité de la lumière) sur la croissance végétative [14]. Mon travail de thèse a aussi été l'occasion de collaborer avec le Professeur Bernard Millet (Laboratoire de Botanique, ISN, Université de Besançon) sur les mécanismes et les caractéristiques de la croissance rythmique endogène [16, 17] qui existe chez un certain nombre d'espèces végétales en l'absence de toute variation environnementale et qui est modulée de manière plus ou moins marquée par l'environnement climatique, qu'il soit tempéré ou tropical. J'ai découvert l'importance des signaux hormonaux (acide abscissique, gibbérellines, cytokinines, …) dans le contrôle de la croissance, et en particulier de l'influence d'organes telles les feuilles sur l'allongement de la tige du *Terminalia superba*

[15]. Mon travail de thèse m'a permis d'acquérir enfin des connaissances en histologie et cytologie sous la houlette de Madame Lucienne Sossountzov (Maître de conférences, Paris VI, [125]). Lors de ma troisième année de thèse en juin 1987, j'ai concouru sur un poste de chargé de recherches à l'INRA, grâce à une dérogation exceptionnelle obtenue par mon directeur de thèse, n'ayant pas encore mon diplôme de doctorat. Le profil du poste était axé sur l'étude du développement végétatif et fructifère d'un arbre fruitier (le noyer, *Juglans regia*). Ayant réussi ce concours et étant devenue chargée de recherches stagiaire INRA en août 1987, j'ai poursuivi la rédaction de mon mémoire [125], soutenu ma thèse en décembre 1987, et reçu l'année suivante la médaille de vermeil de la part de l'Académie d'Agriculture de France pour mes travaux. Ce travail m'a valu une certaine reconnaissance de la part de spécialistes des rythmes biologiques (GERB, France), et de l'architecture des plantes, et donc des discussions intéressantes avec les Professeurs Francis Hallé (Université Montpellier, France), Crabbé (Université de Gembloux, Belgique), Jean-Daniel Viémont (Université d'Angers), et mes collègues François Beaujard (INRA Angers) et Suzanne Lavarenne (Université Blaise Pascal, Clermont-Ferrand) du Groupe d'Etude de l'Arbre (GEA). Une collaboration avec le Professeur Paul Barnola (Université Nancy I), et plus particulièrement une participation à l'encadrement de DEA [132] puis de thèse de Cécile Parmentier [133] m'a permis de partager mes connaissances sur les rythmes de croissance, dans l'étude de l'influence du système racinaire sur la croissance aérienne du chêne pédonculé [20, 83].

En 1988, j'ai effectué un stage d'un an à l'université d'Orsay dans le laboratoire de « Structure et de métabolisme des plantes » des Professeurs Robert Bourdu et Jean-Louis Prioul. Au cours de ce stage je me suis intéressée au métabolisme glucidique du maïs (*Zea mays*), espèce modèle du laboratoire. J'ai à nouveau utilisé l'outil isotopique (^{14}C) pour étudier l'assimilation photosynthétique du carbone (C) par les feuilles matures de maïs. J'ai été initiée à plusieurs techniques utilisées conjointement (chromatographie liquide haute performance, enzymologie, isotopie) et à la modélisation, outil développé par Jean-Paul Rocher (Maître de Conférences, Université d'Orsay). J'ai ainsi pu étudier les flux de C de l'atmosphère vers différents compartiments métaboliques carbonés (glucose, fructose, saccharose, amidon), calculer des vitesses de transfert entre ces compartiments et évaluer les capacités d'exportation de saccharose d'une feuille mature vers les autres parties de la plante [62, 100]. Lors de cette année passée à l'Université d'Orsay j'ai partagé le bureau de deux spécialistes en métabolisme des plantes, mesdames Eliane Deléens et Christine Foyer, avec lesquelles j'ai eu des discussions concernant les métabolismes carboné et azoté et leur inter relations. Madame Eliane Deléens (Directeur de recherches CNRS, IBP, Orsay) m'a alors convaincue de l'intérêt des isotopes stables pour l'étude des flux de carbone (C) et d'azote (N)

dans l'arbre en conditions contrôlées et naturelles, et m'a aidée, avec beaucoup d'enthousiasme et d'amitié, à démarrer mon programme de recherche au laboratoire de Bioclimatologie INRA de Clermont-Ferrand. Ce laboratoire, devenu par la suite UMR PIAF (puis PIAFF) et était spécialisé dans les années 90 dans l'étude de la physiologie des arbres fruitiers en relation avec le climat. Eliane Deléens m'a ouvert grand les portes du monde de l'isotopie stable, aussi bien pour le prêt de matériel, les adresses rares à la fin des années 80 de collègues susceptibles d'analyser mes échantillons (ex. : Hervé Casabianca, CNRS Lyon), que pour l'interprétation des données et l'orientation de ma thématique scientifique. Sur les conseils d'Eliane Deléens, j'ai construit avec l'aide de collègues ingénieurs ma première chambre de marquage isotopique [45] et travaillé sur l'acquisition de l'autotrophie pour le carbone et l'azote du noyer issu de germination. Ce travail s'est réalisé en collaboration avec Eliane et avec mes collègues clermontois François-Alain Daudet, André Lacointe, et Jean-Sylvain Frossard (PIAF). Au cours de cette période j'ai encadré plusieurs étudiants en DEA : Catherine. Roumet [76, 138], Frédéric Castell [9, 35, 111], Didier Chenevard [36, 46, 113] et participé à l'encadrement d'un étudiant en thèse Abdellah Kajji [8, 33, 54, 121]. En collaboration avec Jean-Pierre Gaudillère (INRA, Bordeaux) et François-Alain Daudet, j'ai également participé à l'encadrement d'un post-doctorant Abraham Escobar-Gutierrez [3, 4, 38, 39, 40, 98] dont le sujet portait sur la modélisation des flux de C et d'N dans le jeune noyer, et qui est devenu depuis chercheur à l'INRA de Lusignan. J'ai aussi contribué activement à l'animation d'une AIP ECOFON (Coordinateur Raymond Bonhomme, INRA Guadeloupe) en organisant avec R. Bonhomme deux colloques nationaux suivis de l'édition de deux ouvrages INRA dans la série Colloques [148, 149].

En 1995, j'ai demandé mon détachement à l'INRA de Nancy, dans l'Unité d'Ecophysiologie Forestière pour travailler avec Jean-Marc Guehl (aujourd'hui Chef de Département EFPA). J'ai ainsi pu participer avec mes collègues nancéens au développement des connaissances sur la gestion du C et d'N chez différentes espèces forestières, et la modulation de cette gestion face aux changements climatiques. Philippe Vivin au cours de sa thèse avec J.M. Guehl (thèse soutenue en 1995), avait mis en place une chambre de marquage isotopique en collaboration avec Eliane Deléens et Patrick Gross ingénieur de notre UMR, et utilisé les isotopes stables (^{13}C, ^{15}N) pour ses études. J.M. Guehl avait de plus obtenu le financement d'un spectromètre de masse. Après le départ de Philippe Vivin vers d'autres horizons (Chercheur à l'INRA de Bordeaux), ma thématique, s'est organisée en collaboration avec Jean-Marc Guehl autour de (i) l'étude de la survie de jeunes plants (mélèze, pin laricio, chêne sessile et pédonculé) dans le contexte de la régénération forestière et (ii) de l'impact de changements climatiques (CO_2, O_3, dépôts azotés, sècheresse, …) sur le développement

végétatif et la gestion du C et de l'N d'arbres forestiers (chêne, hêtre) et du peuplier. J'ai alors encadré plusieurs étudiants en DEA sur ces sujets : Didier Garriou [13, 48, 116], Nicolas Barrière [108], Tifenn Pichot [34, 71, 72, 135], et participé à l'encadrement de thèse de Virginie Pellicer sur l'étude des problèmes d'enracinement lors de la propagation par bouturage du mélèze [21, 134].

Le laboratoire de bioclimatologie (Future UMR 1137) était en 1995 spécialisé dans les mesures physiques, et ne possédait pas de laboratoire de biochimie propre. J'ai donc fait en 1997 et 1998 deux demandes d'équipement moyens accordées par le département Forêts et Milieux Naturels qui, couplées à ma dotation d'arrivée à Nancy du département Environnement et Agronomie, m'ont permis d'acheter le matériel nécessaire (ultracentrifugeuse, évaporateur sous vide, spectrophotomètre, appareil à eau ultra pure, lyophilisateur, etc. …) sans lequel l'extraction et le dosage des glucides et d'autres composés (acides aminés, protéines, chlorophylles, …) s'avérait impossible. Ce laboratoire de biochimie appartient désormais au Plateau Technique d'Ecologie Fonctionnelle (PTEF) de notre UMR, dirigé par Nicolas Angeli.

Dès 1997, je me suis rapprochée de mes collègues universitaires, le Professeur Pierre Dizengremel, Dany Afif et Dominique Gérant, Maîtres de conférence et spécialistes du métabolisme carboné et azoté chez les arbres forestiers. Notre première collaboration s'est concrétisée par l'encadrement d'étudiants en DEA (Anne Deschazeaux [29, 114], Yannick Barascud [107]) sur deux sujets concernant l'impact de l'O_3 et du CO_2 sur la croissance et le métabolisme du peuplier et du chêne pédonculé. Les années 2000, et jusqu'à aujourd'hui, ces rapprochements se sont renforcés en particulier avec Dominique Gérant, puis avec notre collègue Nathalie Bréda (INRA Nancy, équipe phytoécologie) très impliquée dans l'étude et la compréhension des phénomènes de dépérissement forestiers à l'échelle de la parcelle et de la région. Et enfin, avec le Professeur Daniel Epron (Directeur adjoint de l'UMR) avec qui Dominique Gérant et moi-même travaillons depuis 2007. Etant donnée l'importance des réserves pour assurer le métabolisme de maintenance des arbres pendant la période hivernale, la croissance du nouveau feuillage au printemps, et la réparation ou à la restauration d'organes endommagés lors de stress biotiques et abiotiques, mes collègues et moi-même avons entrepris de caractériser la gestion saisonnière du C et de l'N d'arbres adultes en situation naturelle sur deux espèces modèle (hêtre, chêne). Nous avons porté un intérêt particulier aux phénomènes de stockage hivernal et de remobilisation printanière des réserves, et aux transferts de C et N dans l'arbre via le phloème. J'ai alors participé à l'encadrement de plusieurs étudiants en thèse : Cécile Barbaroux (j'étais membre de son comité de thèse),

Séraphine Vizoso [25, 141], Luis Valenzuela-Nunes [18, 19, 139], Rana El Zein [5, 6, 27, 115], et enfin depuis 2011 Morgane Pluchon (je suis membre de son comité de thèse).

Activités de recherche

1. Contexte général et problématique scientifique

Les arbres et les forêts couvrent plus de 4,1 x 109 hectares et représentent 30% de la surface terrestre (Dixon *et al.*, 1994). Les arbres représentent un quart du total des plantes vasculaires recensées (env. 400 000 espèces) sur terre, leur hauteur peut dépasser 100 m, et ils sont caractérisés par une longévité exceptionnelle qui peut atteindre 4000 ans (Raven et Crane, 2007). L'importance des arbres pour l'homme se reflète par leur productivité fournissant du matériau bois fortement utilisé (construction, chauffage, etc. …), mais aussi par leur rôle dans le cycle du carbone (Schneider, 1989 ; Dixon *et al.*, 1991 ; Roberntz et Linder, 1999).

1.1. Les arbres face aux changements climatiques

Du fait de leur longévité, les arbres sont soumis aux changements climatiques sur le long terme ce qui met à l'épreuve leurs capacités d'acclimatation. Bien que cette stratégie de longévité caractérisant les ligneux ait nécessité durant l'évolution de fortes capacités d'acclimatation et d'adaptation aux conditions changeantes de l'environnement (Ceulemans et Mousseau, 1994 ; Saxe *et al.*, 1998), les évolutions climatiques rapides prévues pourraient augmenter les risques d'inadaptation et de dépérissement (Chemillier, 1997). Les effets du changement global sur les écosystèmes forestiers ont été d'abord positifs et ont contribué à augmenter leur productivité : (i) augmentation des températures et conséquences sur la phénologie, (ii) augmentation de la concentration en CO_2 atmosphérique et effet positif sur la photosynthèse, et (iii) effet fertilisant associé de l'augmentation des dépôts azotés atmosphériques. Cependant, on peut craindre une augmentation de la vulnérabilité des écosystèmes aux aléas climatiques en particulier (sécheresse, tempête, …) dont la fréquence et l'intensité pourraient également augmenter dans un futur proche. Ainsi, le délai de rentabilité économique et la longue durée de vie des espèces forestières nécessitent une anticipation importante des évolutions climatiques, en tendance et en extrêmes, et de leurs conséquences biologiques, ceci de manière à adapter la sylviculture dès à présent (Bréda *et al.*, 2000 ; Lebourgeois *et al.*, 2001). Les arbres jouent un rôle important dans la séquestration du CO_2 et ainsi contribuent à l'atténuation de l'effet de serre. Certaines estimations suggèrent que l'arrêt de la coupe d'arbres dans les forêts tropicales et le reboisement de 250 millions d'hectares sous les tropiques (ou l'équivalent de 400 millions d'hectares dans les régions tempérées), pourraient séquestrer 25 billions tonnes de carbone dans les prochaines 50 années, ce qui est égal à 1/7ème du total requis pour stabiliser la

concentration du CO_2 atmosphérique à 500 ppm, le niveau actuel étant à 375 ppm (Raven et Crane, 2007).

Pour ce qui concerne l'azote, la pollution automobile, l'industrie et l'élevage sont à l'origine de la majeure partie des dépôts d'azote atmosphérique constatés. Ces apports passifs d'azote minéral se font via les dépôts humides (nitrate NO_3^-, ammonium NH_4^+ et HNO_3) et secs (NH_3 et NO_x). Dans les forêts françaises, la quantité totale d'azote apportée sous forme de dépôts varie en moyenne entre 2,5 à 20 kg N ha^{-1} an^{-1} pour NH_x et entre 2 à 27 kg N ha^{-1} an^{-1} pour le NO_x (Landmann *et al.*, 1991). Dans les régions polluées, les apports d'azote atmosphérique sont parfois supérieurs aux besoins des peuplements forestiers. Ces apports excessifs peuvent avoir des conséquences néfastes telles que la toxicité directe au niveau des feuilles (Skeffington et Wilson, 1988) ou l'acidification du sol (Dambrine *et al.*, 1991).

1.2. Les arbres et le cycle du carbone

Les écosystèmes forestiers fixent par la photosynthèse un bilan net de 1,5 Gt de carbone, représentant ainsi 20% des émissions humaines de CO_2 (Mortier, 1995). Ils constituent le stock de carbone terrestre le plus important et contiennent plus de 60% du carbone stocké dans la biosphère terrestre (van de Walle *et al.*, 2001). Le carbone stocké dans les arbres varie suivant les espèces forestières de 23 à 82% du stock total de carbone de l'écosystème (van de Walle *et al.*, 2001). Les forêts tempérées, par exemple, couvrent environ 10 millions de km^2 (25% de la surface forestière terrestre, IPCC, 1998), et sont des puits de carbone estimés entre 1,4 à 2 tonnes C. $ha^{-1}.an^{-1}$ (Nabuurs *et al.*, 1997 ; Brown et Schroeder, 1999). Ces forêts participent donc de manière importante à la limitation de l'augmentation de $[CO_2]$ terrestre. Une stimulation de l'activité photosynthétique des feuilles, liée à une augmentation de la vitesse de carboxylation ainsi qu'à une diminution de la vitesse d'oxygénation de la Rubisco est à l'origine de l'entrée en excès de C dans les plantes sous $[CO_2]$ élevée (Stitt, 1991 ; Drake *et al.*, 1997), et de la stimulation marquée de la croissance des arbres (Ceulemans et Mousseau, 1994).

1.3. Les arbres et le cycle de l'azote

L'azote, le phosphore et l'eau sont des facteurs connus pour limiter la productivité des écosystèmes forestiers des régions tempérées (Schneider *et al.*, 1996). Dans le sol, l'azote est majoritairement présent sous forme organique (humus principalement) et l'azote inorganique (NH_4^+, NO_3^-) ne représente environ que 1 à 2% de l'azote du sol (Marschner et Dell, 1994). L'azote inorganique, assimilable par les plantes est présent dans le sol sous trois formes principales (NO_3^-, NH_4^+, et l'azote moléculaire N_2 ; Titus et Kang, 1982). La fixation symbiotique de l'N_2 ne concerne qu'un très faible nombre d'espèces arborées (plantes

actinorhiziennes telles que l'aulne *Alnus glutinosa* ou le robinier *Robinia pseudoacacia*). L'acquisition de l'azote par les arbres forestiers a donc lieu essentiellement à partir de NO_3^- et NH_4^+. Peut-être du fait d'une adaptation aux sols forestiers acides généralement pauvres en NO_3^-, une absorption préférentielle du NH_4^+ par rapport au NO_3^- a été rapportée pour le hêtre et le pin par certains auteurs (Carrodus, 1967 ; France et Reid, 1979). De nombreux arbres forestiers ont aussi la capacité d'améliorer leur nutrition minérale lorsque la minéralisation du sol est faible, grâce à la formation de mycorrhizes, organes symbiotiques issus de l'association de racines fines avec un champignon filamenteux (Martin et Botton, 1993). Par ce biais, et en échange de la fourniture de composés carbonés au champignon, l'arbre hôte reçoit des formes d'azote organique (acides aminés, peptides, protéines).

L'azote absorbé par l'arbre est assimilé en acides aminés qui peuvent utilisés pour la synthèse protéique au niveau racinaire ou transportés vers les autres organes de l'arbre. Des études menées sur différentes espèces ligneuses on montré que les acides aminés sont transportés vers les autres organes par le xylème, mais aussi par le phloème, empruntant alors probablement dans ce cas la voie du cambium et des rayons ligneux et phloémiens (Titus et Kang, 1982 ; Dickson *et al.*, 1985).

La synthèse de composés azotés (acides aminés, protéines et acides nucléiques) à partir de l'azote absorbé assure des fonctions essentielles au niveau foliaire pour le fonctionnement de la photosynthèse (chlorophylles, Rubisco, par exemple) et plus généralement pour le fonctionnement métabolique général de l'arbre et le maintien de ses fonctions (respiration, développement, stockage). Dans les forêts décidues tempérées, la chute de la litière à l'automne et sa décomposition par les microorganismes du sol participent de manière essentielle au recyclage des éléments nutritifs des écosystèmes forestiers.

1.4. Interactions entre métabolismes du carbone et de l'azote

Depuis les années 80 et jusqu'à aujourd'hui des études menées sur plantes herbacées ont montré l'existence d'une étroite interaction entre les métabolismes carboné et azoté (Foyer *et al.*, 2001). D'une part, l'assimilation du C nécessite l'intervention de molécules azotées comme la Rubisco et les enzymes du cycle de Calvin, d'autre part, elle mobilise une grande partie de l'azote de la plante, puisqu'une grande fraction du pool azoté est investie dans l'appareil photosynthétique. Dans l'arbre par exemple, les feuilles peuvent contenir de 40 à 50% de l'azote total (Forshey, 1963 ; Dickson, 1989). Enfin, l'absorption et l'assimilation de l'azote nécessitent également des molécules dérivant du métabolisme carboné. Ainsi, l'énergie et le pouvoir réducteur nécessaire à l'absorption et à l'assimilation de l'azote minéral sont fournis par le métabolisme carboné (Salsac *et al.*, 1987 ; Foyer *et al.*, 2001).

A l'échelle de la plante, les interactions entre métabolismes carboné et azoté impliquent une importante co-régulation des activités racinaire et foliaire (Bloom *et al.*, 1993). Si l'activité d'un métabolisme est perturbée, un ajustement se produit dans l'autre (Martins-Louçao et Cruz, 1999 ; Ferrario *et al.*, 2001). L'hypothèse d'un équilibre fonctionnel entre la photosynthèse et l'assimilation de l'azote, de façon à maintenir un rapport C/N relativement stable et compatible avec la vie par exemple, a été formulée par différents auteurs (Davidson 1969 ; Foyer *et al.*, 2001). De même l'interaction entre les métabolismes carboné et azoté implique une régulation de la répartition du C photosynthétique entre la synthèse des sucres et des acides aminés (Champigny *et al.*, 1991). Cette notion d'équilibre fonctionnel est apparue dès les années 60 à partir d'études sur les conditions nutritionnelles (en particulier déficience en azote) et leur impact sur le rapport biomasse racine/biomasse tige de plants (Brouwer, 1962). Ainsi, en conditions hydriques et minérales non limitantes par ailleurs, l'application d'azote est connue pour augmenter la croissance aérienne et donc diminuer le rapport tige/racine des plants traités (Brouwer, 1962, 1983). L'équilibre fonctionnel implique que non seulement les métabolismes carboné et azoté interagissent de façon étroite, mais aussi qu'à moyen terme, des ajustements de la croissance des organes assimilateurs concernés s'opèrent.

Chez les arbres forestiers les avancées concernant les métabolismes carboné et azoté et leurs interactions progressent lentement comparées à celles obtenues sur des plantes d'intérêt agronomique. Cependant, les résultats déjà acquis montrent que les mécanismes métaboliques mis en jeu dans l'arbre présentent des similitudes avec ceux mis en jeu dans les plantes herbacées (Gaudillère, 1997 ; Martin *et al.*, 2001 ; Hoch 2003, Hoch, 2007).

1.5. Mise en réserve du C et de l'N chez les arbres et rythmes saisonniers

La mise en réserve du C (amidon) et de l'N (acides aminés, protéines solubles) par les arbres décidus suit un rythme saisonnier (Hoch, 2003 ; Titus et Kang, 1982; Millard, 1993, 1996). Cette mise en réserve est une des fonctions majeures permettant d'assurer (i) la survie de l'arbre en cas de stress biotique ou abiotique et (ii) sa croissance printanière chaque année (Hoch, 2003).

En climat tempéré, les teneurs en amidon dans les arbres atteignent des valeurs maximales à la fin de l'automne (Lacointe *et al.*, 1994 ; Barbaroux et Bréda, 2002). En automne également, les jours courts et la baisse de température entraînent un recyclage de l'azote depuis les feuilles sénescentes vers les organes pérennes tels que le tronc, les branches, les racines et les pousses (Thomas et Stoddart, 1980). Les protéines foliaires sont dégradées en acides aminés qui seront transportés via le phloème vers les tissus pérennes où les protéines de réserve [Vegetative storage protein (VSP)] seront synthétisées et accumulées

dans le parenchyme de l'écorce et dans les rayons ligneux (Titus et Kang, 1982 ; Sauter *et al.*, 1989 ; Wetzel *et al.*, 1989 ; Stepien *et al.*, 1994 ; Hörtensteiner et Feller, 2002 ; Gessler *et al.*, 2004). La remobilisation de l'azote foliaire commence avec le jaunissement des feuilles et se poursuit jusqu'à leur chute (Sauter et Van Cleve, 1994 ; Bollmark *et al.*, 1999). Le pourcentage d'azote remobilisé pendant la sénescence foliaire va de 40 à 80% (Chapin et Kedrowski, 1983 ; Millard et Proe, 1991 ; Vizoso *et al.*, 2008) vers les parties ligneuses de l'arbre et peut permettre de sauvegarder jusqu'à 2/3 de son contenu en azote (Shim *et al.*, 1972). Les variations saisonnières des réserves azotées suivent une dynamique apparemment plus simple que celle des réserves glucidiques. Cette dynamique se caractérise par 2 phases : une phase d'accumulation dans les organes pérennes à la fin de l'automne et en hiver, suivie d'une phase de remobilisation intense au printemps pour la croissance des nouveaux organes (Titus et Kang, 1982). Le recyclage interne saisonnier de l'azote est en fait lié fortement à la phénologie des arbres (Millard et Grelet, 2010).

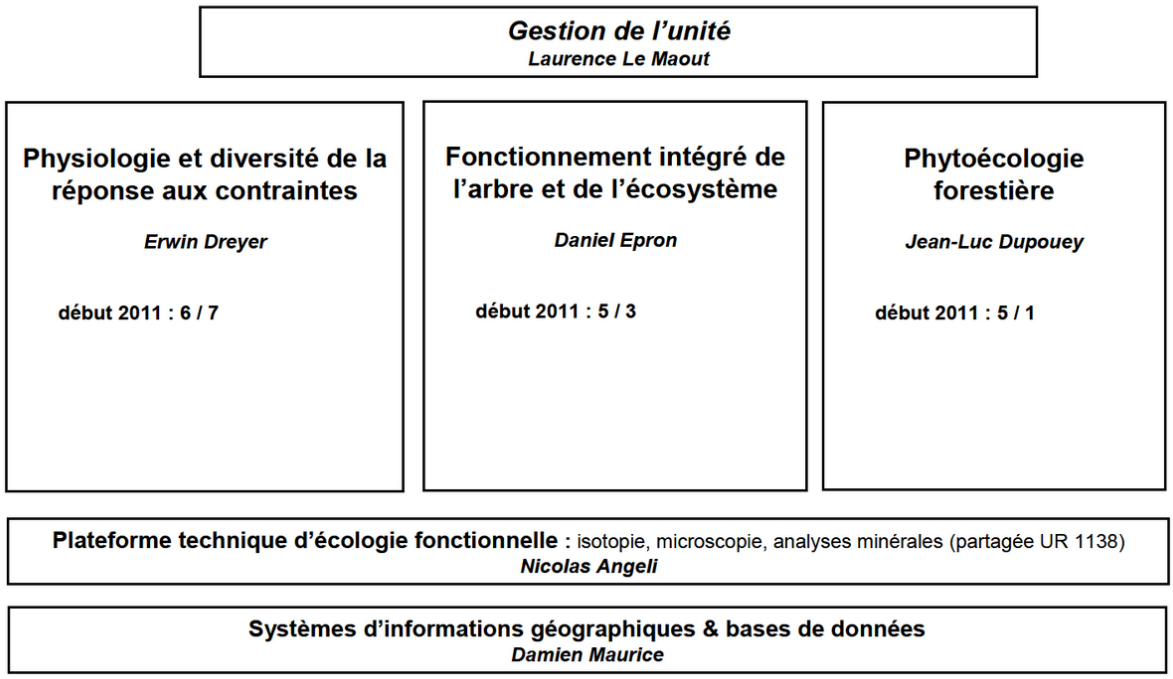
Les arbres entrent ensuite en repos végétatif pendant l'hiver. Pendant cette période de dormance, l'assimilation des ressources est ralentie ou absente. Les arbres assurent alors l'entretien de leurs tissus grâce aux réserves stockées dans leurs tissus végétatifs (Kramer et Kozlowski, 1960). Les réserves permettent aussi aux arbres de s'endurcir au froid et de résister aux gelées hivernales (Sakai et Larcher, 1987 ; Alves *et al.*, 2004 ; Morin *et al.*, 2007). Enfin, les composés de réserve impliqués dans le rythme saisonnier des arbres sont des éléments essentiels dans le maintien de leur intégrité physiologique face aux contraintes climatiques et biotiques pluriannuelles comme la sécheresse (Bréda *et al.*, 2006), les défoliations (Marcais et Bréda, 2006), les attaques d'insectes (Rohde *et al.*, 1996 ; Guérard *et al.*, 2007) et les blessures (Bory *et al.*, 1991). Les réserves et leur concentration dans l'arbre peuvent être aussi utilisées comme indicateurs pour évaluer l'état de santé et la vitalité de l'arbre (Renaud et Mauffette, 1991).

Les connaissances acquises sur la gestion du C et de l'N et en particulier la gestion des réserves (stockage et remobilisation) sont donc fondamentales pour mieux comprendre les dynamiques interannuelles de croissance et les « effets retard » d'épisodes de contraintes climatiques ou biotiques sur la croissance des arbres forestiers pendant plusieurs années successives, voire sur leur mortalité.

2. Bilan scientifique

2.1. Situation au sein de l'Unité Mixte de Recherche (UMR 1137)

L'UMR 1137, à laquelle j'appartiens, travaille sur la diversité des traits fonctionnels des arbres forestiers. Notre UMR s'intéresse particulièrement au fonctionnement des arbres, des peuplements et des couverts forestiers, et à leur rôle dans les cycles du carbone, de l'azote et de l'eau. Les espèces plus particulièrement étudiées sont le chêne, le hêtre et le peuplier. Au sein de notre UMR, les approches de mon équipe (Equipe 2 : ARBECO, fonctionnement intégré de l'arbre et de l'écosystème) visent à intégrer le fonctionnement et l'écologie d'espèces forestières d'intérêt, de l'échelle cellulaire à l'écosystème, et à accéder à des dynamiques intra- et inter- annuelles par le biais d'outils isotopiques et biochimiques. L'UMR dispose pour cela d'équipements de mesures physiques, de spectromètres de masse isotopique ainsi que de tout l'équipement nécessaire aux analyses biochimiques.



Organigramme de l'UMR EEF Pour chacune des trois équipes, le premier chiffre correspond au nombre de chercheurs (DR, CR et IR), le second au d'enseignants chercheurs dans chaque équipe. Les noms indiqués sont ceux des responsables 'équipes et des animateurs de plateaux techniques.

2.2. Situation au sein de l'équipe Arbeco

La thématique centrale de l'équipe ARBECO concerne donc les flux et les bilans d'eau, de carbone et d'azote aux échelles de l'arbre et de l'écosystème. Les principaux axes de recherches qui ont été développés au cours des deux premières années du contrat quadriennal 2009-2012 concernent (1) l'allocation du carbone et de l'azote dans l'arbre, (2) le fractionnement isotopique durant la respiration, (3) les déterminants et la décomposition des flux dans les écosystèmes forestiers et peu anthropisés, (4) la régulation des transferts

hydriques à l'échelle de l'arbre et du peuplement en réponse à la sécheresse édaphique, et (5) les cycles biogéochimiques et le maintien de la fertilité au sein des plantations forestières à courtes rotations. Les activités de mon équipe impliquent l'utilisation de méthodes modernes de mesures des flux, de traçage isotopique, de caractérisations biochimiques et de modélisation. Notre équipe a été notée A lors de la dernière évaluation AERES (Février 2008) et vient d'être évaluée à nouveau positivement en février 2012.

Mon domaine d'étude est l'écophysiologie de l'arbre et mes travaux sont centrés depuis le début des années 90 sur la gestion du carbone et de l'azote, avec un intérêt marqué, bien que n'étant pas modélisatrice, pour la modélisation des flux de C [4 ; 37 ; 62 ; 98 ; 123]. Le volet de recherche, auquel mes travaux contribuent, concerne l'étude des réserves dans les arbres. Ce volet a été jugé essentiel par les évaluateurs de notre UMR, dans la détermination des flux et des pools à l'échelle de l'écosystème pour deux raisons principales: (1) en complément des mesures de flux, il est nécessaire d'évaluer les différentes composantes du puits de carbone dans les forêts pour déterminer la dynamique du cycle de carbone en particulier dans le contexte de l'augmentation de la teneur de l'atmosphère en CO_2; (2) ces réserves représentent une information essentielle du potentiel de séquestration des forêts en zone tempérée.

En 2002 Barbaroux et Bréda avait montré que les concentrations en glucides non structuraux de l'aubier du tronc, et ses variations saisonnières, sont plus importantes chez le chêne sessile que chez le hêtre. Les différences observées ont été attribuées en toute hypothèse à l'anatomie du bois contrastée des deux espèces (bois poreux versus diffus) et à leurs schémas de croissance radiale et de débourrement au printemps différents. A la suite de cette étude, Nathalie Bréda (DR2, Equipe 3 Phytoécologie Forestière), Dominique Gérant (MC, Equipe 2 ARBECO) et moi-même avons décidé d'approfondir ensemble nos connaissances sur les dynamiques saisonnières des réserves carbonées sur ces deux espèces au stade adulte. Nous avons développé en particulier des recherches sur les réserves azotées par le biais du co- encadrement de deux thèses [115, 139].

Nos objectifs sont, à terme, d'évaluer l'impact de l'environnement biotique et abiotique sur le stockage et la remobilisation des réserves et sur le développement des arbres adultes. Nos approches incluent des mesures de croissance, de la biochimie classique (glucides, acides aminés, protéines solubles totales), des approches métaboliques et moléculaires [activités enzymatiques, caractérisation et séquençage de VSP] dont Dominique Gérant est la spécialiste, et des marquages isotopiques (^{13}C, ^{15}N) dont j'ai l'expertise.

Au sein de l'UMR, nos compétences complémentaires sont un atout certain pour accéder à une compréhension intégrée de la physiologie des arbres et des peuplements, et

grâce à ce rapprochement de compétences fructueux, la composante « réserves » est dorénavant intégrée de manière importante aux thématiques de l'UMR.

2.3. Activités principales de recherche (période 2005-2011)

Mon activité de recherche principale depuis 2005 vise à une meilleure compréhension de la gestion du C et de l'N, et de l'importance des réserves carbonées et azotées pour assurer la survie de l'arbre face (i) aux changements climatiques (CO_2, O_3, dépôts azotés, sécheresse, défoliations,…) et (ii) aux problèmes de croissance racinaire et aérienne lors de la transplantation en forêt française et méditerranéenne. Mes outils sont principalement (i) l'isotopie stable qui me permet de suivre par marquage l'impact de l'environnement sur l'entrée et la répartition du C et de l'N assimilés par l'arbre, des organes assimilateurs (feuilles, racines) aux organes puits ; et (ii) la biochimie des glucides (sucres solubles, amidon) et des composés azotés (acides aminés, protéines solubles totales) qui me permet d'apprécier le devenir du C et de l'N dans les compartiments biochimiques solubles et de réserve. J'ai participé à ce titre, à des études menées, par mes collègues Dany Afif et Mireille Cabané (Equipe 1 UMR EEF), sur le métabolisme des lignines et la formation de bois de tension chez le peuplier en relation avec le CO_2 et l'O_3, et ai contribué dans le cadre d'une expérience de marquage au travail de thèse de Nicolas Richet [23, 84, 85, 136].

J'ai aussi collaboré ces dernières années avec quelques collègues à l'étranger (Pedro Villar Salvador, Espagne ; Andrew Merchant, Post-doctorant Australie) et ai apporté mes compétences afin d'améliorer les connaissances sur le fonctionnement carboné et azoté de différentes espèces méditerranéennes (olivier, pin d'alep, chêne vert, chêne kermes) en réponse à la transplantation, et du métabolisme du quercitol foliaire de l'eucalyptus en réponse à la sécheresse estivale [collaboration avec D. Gérant et Erwin Dreyer (Equipe 1)].

Bien que m'éloignant des préoccupations forestières de notre UMR, et suite à une demande de collaboration de mes collègues de l'université de Reims (Florence Fontaine et Nathalie Gaveau), je me suis intéressée à l'étude des relations source puits pour le C de boutures fructifères de vigne Chardonnay au cours du développement de la grappe. Ce travail a été l'occasion de participer à l'encadrement de Geneviève Wojnarowiecz en thèse à l'université de Reims [24, 94, 95].

Par le biais de collaborations intra-UMR et hors UMR (Professeur Michel Chalot et Dr Jean Garbaye, UMR IAM) et de projets régionaux et nationaux dont le financement a été acquis pour des durées de 1 à 4 ans (projet région Lorraine émergent, projets innovants INRA, projets ANR CATS et Intens&Fix), mes recherches qui étaient majoritairement axées sur l'étude de jeunes plants en pépinière ou en serre, s'orientent davantage vers l'étude de la compréhension des flux C-N-H_2O chez les arbres adultes en situation naturelle, en incluant

dans ces études les aspects symbiotiques (chêne) et de fixation d'azote (robinier). Et donc, bien que je m'intéresse toujours au fonctionnement de jeunes plants, aux relations source-puits pour le carbone et l'azote, et aux problèmes de régénération forestière artificielle, l'importance que j'accorde à l'étude du fonctionnement d'arbres adultes en situation naturelle est croissante.

2.4. Etude de l'impact des changements climatiques sur la croissance des arbres jeunes

2.4.1. Effets combinés d'une fertilisation azotée et d'un doublement de la teneur en CO_2 sur la gestion du carbone et de l'azote chez le chêne pédonculé

La disponibilité en azote du sol peut induire des modifications du stockage et de la remobilisation des réserves chez les arbres forestiers, et affecter leur croissance et leur teneur en azote en réponse à l'augmentation du CO_2 atmosphérique (Drake *et al.*, 1997). Cependant, la plante piloterait davantage sa concentration en azote et donc sa demande vis-à-vis du sol que la disponibilité en azote du sol elle-même selon Cotrufo *et al.* (1998).

Nous avons donc analysé l'effet d'une fertilisation azotée sur des plants de chêne pédonculé exposés pendant deux ans à une teneur en CO_2 ambiante ou double en combinaison avec un faible (0,61 mmol N. L^{-1}) et un fort apport d'azote (6,1 mmol N. L^{-1}) dans la solution nutritive du sol. Les résultats montrent que la récupération de l'azote des feuilles par les plants avant leur chute à l'automne a été moins importante dans les plants bien fertilisés que dans les plants carencés en azote. Cependant, environ 15% de l'azote présent dans le plant étaient retrouvés dans la litière quel que soit le traitement appliqué. Pendant l'hiver, les sucres non structuraux représentaient de 20 à 30% des la biomasse des plants. L'amidon était stocké principalement dans les grosses racines et sa concentration a été augmentée par le déficit en azote, sans effet du CO_2. La fertilisation azotée a permis l'augmentation des teneurs en composés azotés solubles, mais le CO_2 a diminué partiellement cet effet. Aussi, les plants fertilisés poussant sous CO_2 élevé ont vu leurs teneurs en acides aminés et en protéines solubles diminuées (-37 et -17 % respectivement) comparativement à ceux cultivés sous CO_2 ambiant. Nous avons marqué les réserves carbonées et azotées à l'aide de ^{13}C et de ^{15}N. Ce marquage nous a permis de montrer que 20% du carbone marqué et 50% de l'azote marqué est remobilisé au printemps, ceci quel que soit le traitement appliqué (N, CO_2). Ce résultat est lié à la forte implication des composés de réserve lors de l'édification des nouveaux organes (Figure 1), et donc à une indépendance du plant vis-à-vis des conditions du milieu tant qu'il est hétérotrophe pour le carbone et l'azote.

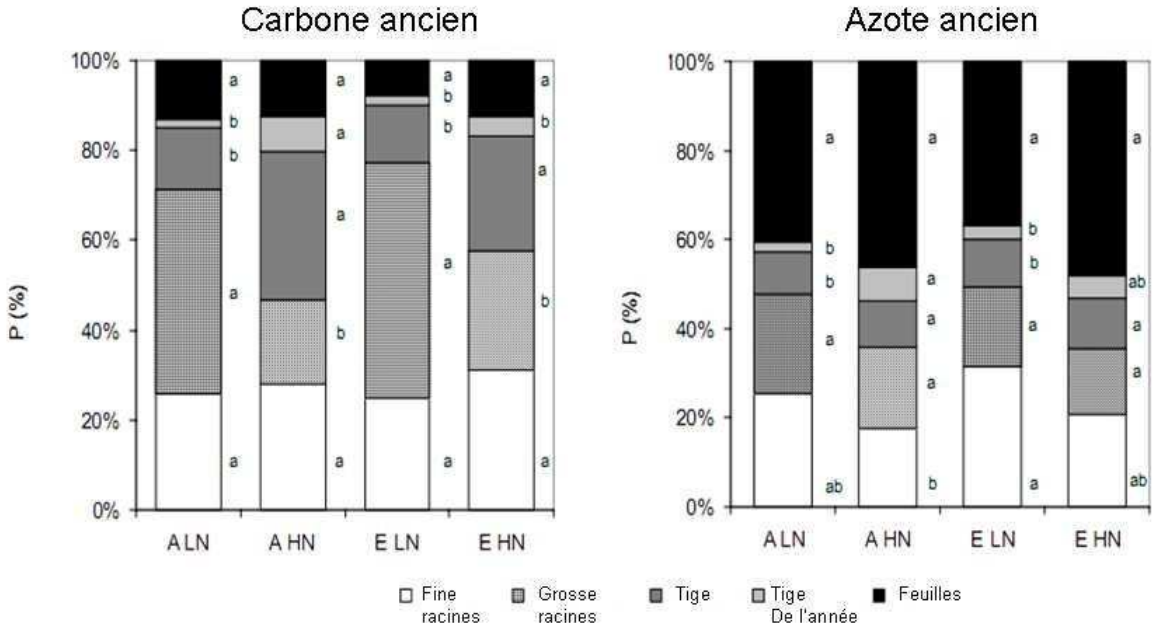


Figure 1. Proportion de C ancien (C) et d'N ancien (D) dans les différents organes de jeunes chênes (*Quercus robur* L.) après expansion complète de la nouvelle pousse. Les plants ont été exposés pendant 2 ans à 350 (A) ou 700 (E) µmol mol^{-1} CO_2 et à deux niveaux de fertilisation azotée (HN 6.1; LN, 0.61 mmol l^{-1}). La récolte a été faite en Avril lors de la 2ème année de croissance (Tiré de la thèse de S. Vizoso [141]).

Le rôle prépondérant de l'azote, mis en réserve à l'automne, sur la reprise de croissance des nouveaux organes, le printemps suivant, a été mis en évidence. En fin de croissance foliaire, 50 à 60% du carbone provenait de l'assimilation photosynthétique contre 10 à 20% dans les plants carencés en azote. Une absorption d'azote par les racines a été notée uniquement pour les plants fertilisés et poussant sous CO_2 double. Par leur action stimulante sur l'accumulation de biomasse et de réserves C et N dans les organes pérennes (tige, racine) avant l'hiver, la [CO_2] double et la fertilisation ont permis au plants de disposer de plus de C et d'N pour réaliser leur croissance printanière l'année suivante. Les plants de chêne ont notamment formé plus de branches et de feuilles, induisant une assimilation de C accrue, ce qui présuppose un effet rétro actif positif des conditions de croissance favorables passées (N, CO_2) d'une année sur la suivante [25, 141].

2.4.2. Effets de l'ozone et de la concentration en CO_2 sur l'allocation du ^{13}C et la synthèse de lignine dans le bois de tension et opposé de plants de peuplier

L'un des objectifs du travail de thèse de Nicolas Richet était de comprendre sur une espèce modèle (le peuplier) comment, dans le contexte des changements climatiques globaux, la croissance des arbres, la formation du bois et sa composition biochimique vont être impactées par la présence simultanée de fortes teneurs en CO_2 et de pics d'ozone. En effet, peu de d'informations sont disponibles concernant l'effet des deux polluants combinés sur l'anatomie du bois et sa composition chimique (Kostiainen *et al.*, 2008). Les résultats de Nicolas Richet ayant suggéré que l'ozone induisait une baisse de synthèse de lignine du fait

d'un défaut d'allocation du carbone vers les tiges, nous avons discuté d'une expérience de marquage au $^{13}CO_2$ sur les plants qui permettrait d'analyser l'allocation du carbone photosynthétique vers le bois et la lignine.

J'ai réalisé avec Nicolas Richet et Mireille Cabané une expérience de marquage ^{13}C en hiver 2008 sur des plants de peupliers soumis à différentes fumigations d'O_3 et de [CO_2] atmosphérique, combinées ou non. De jeunes plants de peupliers inclinés à 42° ont été placés en chambres phytotroniques en présence d'ozone seul (200 nL L^{-1}, 9h-22h), de fort CO_2 seul (800 ppm) ou d'ozone combiné au fort CO_2 [respectivement 200 nL L^{-1} (9h-22H) et 800 ppm] pendant 60 jours. L'objectif principal a été d'analyser l'impact de ces polluants sur la répartition du C photosynthétique dans les parties aériennes, et en particulier dans le bois de tension (RT) et opposé (BO) de la tige. Précisément, l'impact des fumigations sur la synthèse des lignines dans la tige a été recherché en analysant l'intensité de marquage ^{13}C dans les lignines extraites et purifiées par B. Pollet et C. Lapierre (AgroParisTech, UMR 206 Chimie biologique).

Les résultats d'analyse sont très intéressants car ils montrent bien le défaut d'allocation de carbone marqué vers les tiges, en présence d'O_3 (Tableau 1, à mettre en parallèle avec la baisse observée des échanges gazeux dans les feuilles) et confirment bien les hypothèses de départ. Les résultats suggèrent que les différences d'activité métabolique entre BT et BO s'estompent sous ozone ce qui est en accord avec les résultats d'infra-densité, de teneur en cellulose et lignines et l'épaisseur des parois. Par ailleurs, les résultats suggèrent une réponse particulière en présence des deux traitements, ozone et fort CO_2. L'analyse ^{13}C de la lignine (C. Lapierre, C. Bréchet, PTEF) a permis de déterminer l'impact de l'O_3 et du CO_2 sur la source de carbone utilisée pour sa synthèse (photosynthèse, réserves). Notre étude a montré une baisse du carbone alloué à la lignine dans le BT, supposant une répartition en faveur des autres composés du bois, probablement la cellulose afin de produire une quantité de bois de tension plus importante pour un redressement plus rapide de la tige [23, 84, 85, 136].

Tableau 1. Fraction du carbone assimilé lors du marquage ^{13}C dans le carbone total (Relative specific allocation en % ou RSA) de la tige, au niveau du bois de tension et opposé. 35 jours de fumigation à l'ozone et au CO_2. Les données correspondent à la moyenne pour 5 plants ± SD. * représente une différence significative par rapport aux plants témoin non traités à p <0,05 (tiré de la thèse de N. Richet [136]).

		Bois de tension				Bois opposé			
		Control	Ozone	CO2	Ozone-CO2	Control	Ozone	CO2	Ozone-CO2
Tige du milieu	RSA %	2.33 ±0.28	1.24 ±01.5*	2.93 ±0.37	3.15 ±0.21*	2.24 ±0.21	1.10 ±0.07*	2.48 ±0.37	2.59 ±0.12
Tige basse	RSA %	1.78 ±0.13	0.98 ±0.12*	2.21 ±0.20*	1.54 ±0.13	1.57 ±0.17	0.97 ±0.11*	1.65 ±0.21	1.41 ±0.15

2.5. Ecophysiologie de la production et de la transplantation des plants forestiers

2.5.1. Effet d'une défoliation estivale partielle et de l'ombrage sur la croissance printanière de chênes sessile et de hêtre âgés de trois ans

En régénération forestière naturelle, les arbres jeunes sont rarement placés dans des conditions de développement optimal parce que certains facteurs écologiques comme la lumière peuvent être limitants. De plus, ces arbres doivent faire face aux facteurs biotiques présents, et à l'attaque ou la perte de feuilles (défoliation) causées par des maladies, des insectes ou le broutage par des herbivores. Ces attaques foliaires vont se traduire par une diminution de la photosynthèse (Thomas *et al.*, 2004 ; Nykänen et Koricheva, 2004), pénalisant l'entrée de C et donc la croissance (Gerhardt, 1998), et les réserves (Nykänen et Koricheva, 2004). L'existence d'un cycle interne de l'azote alternant stockage et remobilisation de l'azote, joue un rôle important dans le métabolisme, l'assimilation de carbone par le feuillage et la croissance saisonnière des arbres (Millard, 1996). Les feuilles, en tant que réservoirs importants d'azote, et en tant qu'organes assimilateurs de carbone sont des organes vitaux pour l'arbre. Leur perte par défoliation avant la fin de la croissance saisonnière est susceptible de créer des carences en carbone et en azote et de diminuer le stock de réserves nécessaires pour survivre l'hiver et reconstituer le feuillage au printemps suivant. Etudier l'impact d'une défoliation et de l'ombrage est donc très important pour la gestion sylvicole de jeunes plants forestiers particulièrement sensibles aux stress biotiques et abiotiques aussi bien en reforestation artificielle que naturelle.

Dans une étude menée dans le cadre de la thèse de Luis Valenzuela Nunes [30, 70, 139], les effets d'une défoliation partielle estivale (70% des feuilles ôtées) combinée ou non à un ombrage (66% de l'éclairement incident) sur la croissance et les teneurs en composés C et N de deux espèces à exigence lumineuse contrastée ont été recherchés. Le chêne sessile est une espèce dite héliophile, et le hêtre, une espèce tolérante à l'ombrage (ONF, 1997).

Les résultats de cette étude montrent qu'après défoliation, les plants des deux espèces ont reformé avant l'hiver des feuilles en nombre à peu près équivalent à celui des plants contrôle mais de plus petite taille (Figure 2). De plus, l'accumulation de biomasse dans les organes de stockage (tige, grosses racines) a été négativement impactée par la défoliation [139]. Le printemps suivant, un marquage en ^{15}N ($^{15}NH_4$, $^{15}NO_2$, 2 atomes %) des pots a permis de suivre l'absorption minérale du débourrement à l'étalement complet du nouveau feuillage. Cette absorption minérale a permis de renouveler de 5 à 20% de l'azote total du plant selon les conditions d'éclairement et de défoliation appliquées, ceci trente jours après débourrement. L'absorption minérale a donc joué un rôle non négligeable dans la croissance

printanière. Néanmoins, les plants défoliés des deux espèces ont vu leur stock total d'azote diminué quantitativement par la défoliation de l'année passée et l'absorption minérale printanière n'a pas permis de compenser cette perte (Figure 3 a, b).

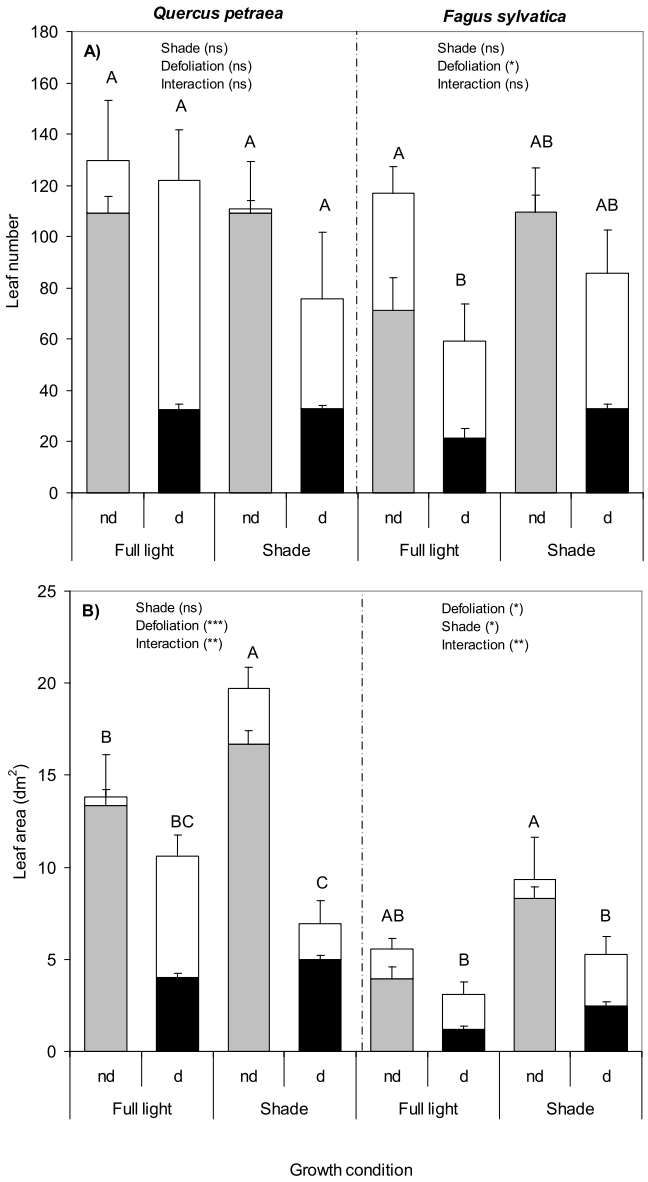


Figure 2. Effet d'une défoliation partielle estivale (70% of leaves) sur le nombre et la surface des feuilles reformées par des plants *Quercus petraea* et *Fagus sylvatica* la même année. Les plants se sont développés du semis jusqu'à l'âge de deux ans en pépinière en pleine lumière ou sous ombrage (66% de la lumière incidente). Chaque valeur est la moyenne de 6-7 plants ± erreur standard. Les lettres différentes indiquent des différences significatives entre les traitements. Les étoiles indiquent des différences significatives (ANOVA double facteur, à $P < 0.05$): ns, non-significatif; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (article en préparation [30]).

En ce qui concerne la concentration, ni l'azote total, ni les acides aminés et les protéines solubles des plants défoliés n'ont été impactés de façon notable au printemps par la défoliation de l'année précédente (Tableau 2). Ces résultats suggèrent donc qu'en cas de perte de carbone et d'azote par défoliation, les plants ajustent leur croissance en fonction des stocks disponibles (quantités C et N diminuées) mais ne modifient pas drastiquement leur métabolisme (pas de dilution notable d'azote, ou de compensation de la perte d'azote par une

absorption minérale accrue par exemple). Le seul effet défoliation détecté a été une augmentation légère chez le hêtre des concentrations en sucres non structuraux.

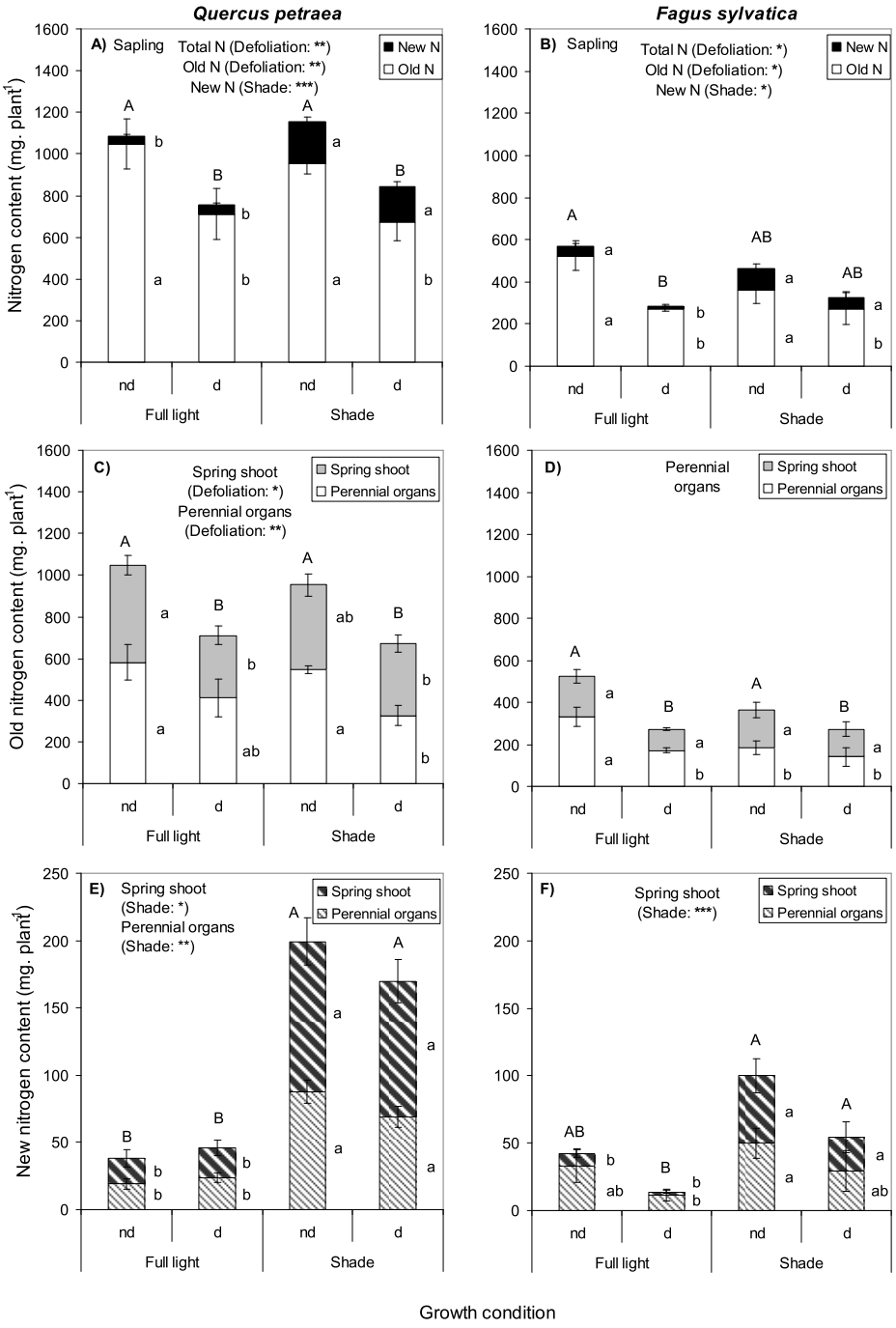


Figure 3. Effets de l'ombrage (65 % de la lumière incidente) et d'une défoliation estivale partielle (70% des feuilles) sur les quantités d'azote ancien et nouvellement absorbé au printemps suivant, en fin de première vague de croissance chez des chênes pédonculé et des hêtres âgés de trois ans. Chaque valeur est la moyenne de 5 échantillons ± erreur standard. A à B : Azote ancien et nouveau dans le plant entier ; C à D : Azote ancien réparti entre les nouvelles pousses et les organes pérennes ; E à F : Azote nouveau réparti entre les nouvelles pousses et les organes pérennes. Organes pérennes = racines et tronc. Azote ancien : azote de l'année précédente ; Azote nouveau : azote absorbé au printemps. Les lettres différentes indiquent des différences significatives entre les traitements. Les étoiles indiquent des différences significatives (ANOVA double facteur à $P < 0.05$): ns, non-significatif; *$P < 0,05$; **$P < 0,01$; ***$P < 0,001$ (article en préparation [30]).

Tableau 2. Effets de l'ombrage (66 % de la lumière incidente) et d'une défoliation estivale partielle (70% des feuilles) sur les concentrations en sucres solubles non structuraux (NSC) et en composés azotés solubles non structuraux (NSNC) de chênes pédonculés et de hêtres âgés de trois ans. Données obtenues au printemps en fin de première vague de croissance. Chaque valeur est la moyenne de 6 ou 7 plants ± erreur standard. Les lettres différentes indiquent des différences significatives entre les traitements. Les étoiles indiquent des différences significatives (ANOVA double facteur à P < 0.05): ns, non-significatif; *P < 0,05; **P < 0,01; ***P < 0,001 (Tiré du master 2 de D. Konaté [122]; article en préparation[30]).

		Concentration (g. 100 g^{-1} poids sec)						
						ANOVA		
		Pleine lumière		Ombrage				
		Contrôle	Défolié	Contrôle	Défolié	Ombrage (O)	Defoliation (D)	O x D
Quercus petraea								
Plant entier	Sucres Solubles	7,7 ± 0,5	6,9 ± 0,4	8,6 ± 1,2	7,4 ± 0,8	ns	ns	ns
	Amidon	11,2 ± 0,6 ab	12,9 ± 1,2 a	8,9 ± 0,8 b	8,9 ± 0,8 ab	**	ns	ns
	NSC	18,9 ± 0,6	19,2 ± 1,1	17,4 ± 1,7	16,3 ± 1,3	ns	ns	ns
	Acides aminés	0,6 ± 0,1ab	0,4 ± 0,1b	1,0 ± 0,2a	0,9 ± 0,2 ab	**	ns	ns
	Protéines solubles	1,1 ± 0,1 a	1,2 ± 0,1 a	0,8 ± 0,1 ab	0,8 ± 0,0 b	**	ns	ns
	NSNC	1,7 ± 0,2	1,6 ± 0,09	1,9 ± 0,2	1,7 ± 0,2	ns	ns	ns
Fagus sylvatica								
Plant entier	Sucres Solubles	4,7 ± 0,7 b	7,3 ± 0,5 ab	7,0 ± 0,4 ab	8,4 ± 0,2 a	**	***	ns
	Amidon	9,3 ± 1,5	9,5 ± 0,9	6,6 ± 0,3	6,3 ± 0,4	**	ns	ns
	NSC	14,0 ± 1,1	16,7 ± 0,8	13,6 ± 0,4	14,7 ± 0,3	ns	*	ns
	Acides aminés	0,4 ± 0,1	0,37 ± 0,1	0,4 ± 0,1	0,4 ± 0,0	ns	ns	ns
	Protéines solubles	0,3 ± 0,1	0,5 ± 0,1	0,5 ± 0,1	0,4 ± 0,1	ns	ns	ns
	NSNC	0,7 ± 0,1	1,2 ± 0,4	0,9 ± 0,1	0,8 ± 0,1	ns	ns	ns

Quant à l'ombrage il n'affecte pas les concentrations en composés azotés solubles chez le hêtre et le chêne, bien qu'une augmentation d'acides aminés et une légère diminution des protéines solubles soient notées [109, 122]. L'ombrage aboutit à une diminution de la teneur en amidon pour les deux espèces [122]. Ces résultats suggèrent que l'ombrage, par la stimulation de l'allongement de la tige qu'il occasionne pour les deux espèces, solliciterait davantage les réserves C et N et leur remobilisation qu'en pleine lumière, et stimulerait l'absorption minérale et l'allocation accrue de ce nouvel azote vers les nouveaux organes (Figure 3). Notre étude a montré aussi que le chêne et le hêtre âgés de trois ans ne montrent pas de remobilisation quasi-totale de leurs réserves azotées, la part d'azote ancien restant très importante et la concentration en azote total diminuant peu dans les organes de stockage. De plus, le marquage ^{15}N montre bien que la perte causée par l'azote exporté des organes pérennes source vers les nouvelles pousses, est au moins partiellement compensée par l'arrivée de nouvel azote dans les organes pérennes (Figure 3) ce qui suggère un restockage rapide par ces organes (Figure 3). Ce résultat diffère des observations de Millard (1996) qui montre des remobilisations très intenses voire quasi totales dans les organes de stockage des arbres fruitiers.

Le hêtre s'est mieux développé à l'ombre qu'en pleine lumière ce qui correspond bien à ses caractéristiques d'espèce d'ombre. En revanche, nos résultats en termes de croissance, ou de teneurs en C et N, dans les conditions expérimentales de notre étude, remettent un peu en cause le fait que le chêne sessile jeune ait une préférence marquée pour la pleine lumière [139]. Des différences qualitatives de comportement ont pu être notées entre les deux espèces

pour les différentes variables étudiées. Cependant, nous ne pouvons conclure qu'il y a des différences interspécifiques marquées quant au renouvellement de l'azote au sein de jeunes plants bien fertilisés. Des expériences de marquage menées sur ces deux espèces au stade adulte et sur sol forestier pauvre permettrait de comprendre si le renouvellement important de l'azote observé au printemps est lié à la juvénilité ou non [139].

2.5.2. Importance des réserves carbonées et azotées dans la reprise de croissance d'espèces méditerranéennes à la plantation

Le Dr Pedro Villar Salvador, chercheur espagnol du Centro Nacional de MejoraForestal "ElSerranillo" (DGCONA, Ministerio de Medio Ambiente Apdo.249, 19004 Guadalajara, SPAIN) avait obtenu de son ministère une bourse de trois mois en 2004 pour venir collaborer avec moi et se former aux techniques de marquage isotopique (^{13}C, ^{15}N) au travers d'un projet intitulé : « Comparaison interspécifique de la reprise de croissance printanière du pin d'alep, du chêne vert, du chêne kermes et de l'olivier (*Pinus halepensis*, *Quercus ilex*, *Quercus coccifera* et *Olea europaea*) ». L'objectif de l'étude était de d'évaluer l'importance de la remobilisation du C et de l'N stockés comparée à l'utilisation des mêmes éléments nouvellement assimilés, pour assurer la croissance aérienne et racinaire de ces espèces après plantation. L'évaluation de la contribution des deux sources pour chaque élément a été appréhendée en utilisant un double marquage ^{13}C ^{15}N (Schéma 1). La partie expérimentale de cette étude a été réalisée pendant le séjour de mon collègue à Nancy. Puis, cette étude est restée en attente faute de moyens jusqu'en 2009, où le financement des analyses isotopiques et des frais de déplacement et de séjour à Nancy de Mercedes Uscola, étudiante espagnole en thèse avec Pedro Villar Salvador, ont pu être obtenus. J'ai alors encadré Mercedes Uscola de juin à septembre 2009 sur ce sujet.

La nouvelle croissance est essentielle pour l'établissement des semis dans les plantations forestières. Les travaux de Millard montrent que réserves azotées contribuent à hauteur de 50 à 80% dans l'édification des nouveaux organes. Alors que les espèces à feuilles caduques utilisent majoritairement les réserves C stockées pour la croissance, les espèces sempervirentes utilisent aussi les photoassimilats [1, 13, 21]. Cependant, nous avons peu de connaissances sur la façon dont les espèces méditerranéennes à feuilles persistantes utilisent les réserves pour la croissance de leurs organes à la transplantation. Nous avons donc déterminé l'importance relative des réserves C et N pour la croissance rapide des racines et des pousses chez les quatre espèces ligneuses méditerranéennes à feuilles persistantes sus-nommées (*Q. ilex, Q. coccifera, P. halepensis et O. europaea*).

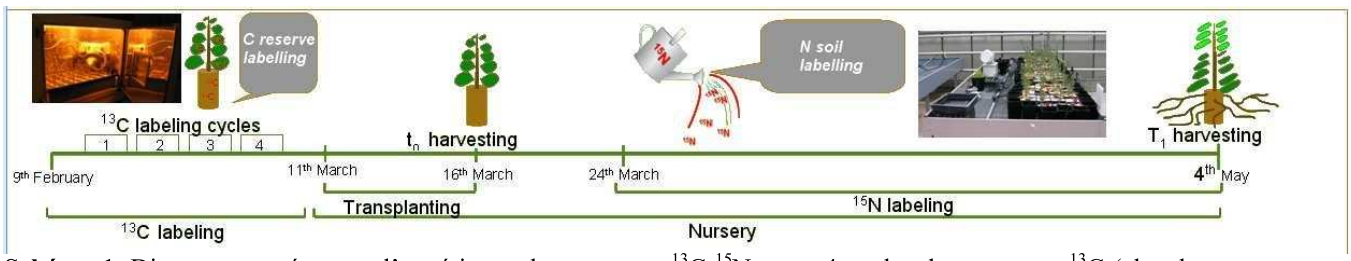


Schéma 1. Diagramme présentant l'expérience de marquage ^{13}C ^{15}N, avec 4 cycles de marquage ^{13}C (chambre de marquage) de février à mars, suivis de la transplantation des plants en serre de culture (mars) puis marquage ^{15}N de la solution du sol jusqu'à la fin de l'expérience (mai, tiré de [86] article en préparation).

Nous avons marqué les réserves C de la plante avec du $^{13}CO_2$ (4 atomes %) et l'azote du sol avec du $^{15}NO_3$ (2 atomes % ; Schéma 1) de façon à distinguer la contribution du C de réserve et l'N nouvellement absorbé dans l'édification des nouveaux organes (pousses, racines fines). Dans l'ensemble, l'utilisation des réserves C et N chez toutes les espèces suivaient la même tendance, mais des variations en fonction de l'organe en croissance ont été observées (Figure 4). Quelle que soit l'espèce, les nouvelles pousses ont davantage utilisé les réserves C et N pour se former que les racines. *Quercus coccifera* est l'espèce qui puisait le moins dans ses réserves pour assurer la croissance des racines et des pousses (<25%).

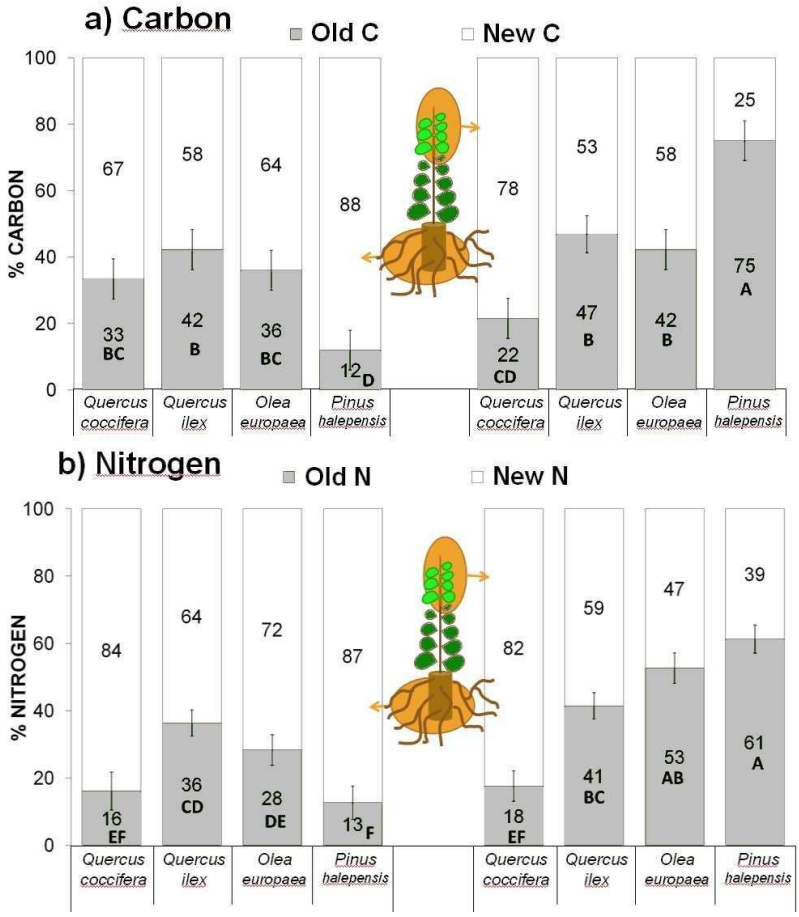


Figure 4. Fractions de C et d'N issus des réserves et nouvellement assimilé dans le C (a) et l'N totaux (b) des parties racinaires (graphes de gauche) et aériennes (graphes de droite). Tiré de [86].

Quercus ilex et *O. europaea* utilisaient en proportion équivalente les réserves et les nouveaux assimilats pour construire leurs nouveaux organes. En revanche, *P. halepensis* montrait un comportement contrasté concernant l'utilisation de ses réserves. Alors que dans les racines moins de 20% du C et de l'N provenaient des réserves, plus de 60% de C et N des nouvelles pousses provenaient de réserves. Les résultats suggèrent que les conditions de culture en pépinière doivent être optimisées (lumière, fertilisation) de façon à permettre un stockage important du C et de l'N par ces espèces, et donc faciliter la croissance des nouveaux organes après leur transplantation en forêt méditerranéenne. Les conditions climatiques et édaphiques après transplantation auront aussi leur importance chez *Q. coccifera*, cette espèce semblant plus dépendante des ressources externes pour assurer sa reprise de croissance que les autres espèces de l'étude. Une communication a été présentée en 2011 dans un congrès international [86] et un article est en préparation.

2.5.3. Importance de la mycorrhization sur les statuts hydrique, carboné et azoté de plants de chêne sessile transplantés en forêt de Mondon

L'UMR IAM (Jean Garbaye) et notre UMR EEF, se sont penchées lors d'un projet régional après-tempête (Forbois) sur l'itinéraire technique pépinière-plantation à parfaire afin d'améliorer la qualité des plants, et favoriser ainsi la reconstitution des hêtraies et chênaies de Lorraine (Collaboration Equipe 1 avec Marie-Béatrice Triboulot-Bogeat ; DEA Emilie Maréchal). Deux paramètres essentiels ainsi que leurs interactions ont été étudiés sur la reprise de croissance en forêt:

- Le statut symbiotique des racines : les arbres forestiers sont très dépendants des champignons mycorhiziens qui leur assurent une nutrition minérale optimale. Dans cette optique, 2000 glands de chêne sessile (*Quercus petraea*) ont été mis à germer, puis inoculés, soit par la souche *Paxillus involutus*, soit par *Scleroderma citrinum*, soit non inoculés.

- Les conditions de conservation des plants : il s'agissait de produire des plants de chêne sessile inoculés ou non par une souche sélectionnée de champignon mycorhizien, et de les conserver avec ou sans sac dans une chambre froide avant de les replanter en forêt (Schéma 2).

Tout au long de la filière pépinière-plantation, pendant trois années, ces plants ont fait l'objet d'un suivi rigoureux par les deux UMR : Le taux de survie, la croissance, l'évolution des mycorhizes, ainsi que le suivi du fonctionnement hydrique et de l'assimilation carbonée des plants ont été réalisés. Ce travail, et le suivi écophysiologique de plants de chêne sessile au cours d'un stockage en chambre froide, puis après transplantation en forêt, avait pour but

d'étudier les effets de deux paramètres : mode de stockage et mycorhization contrôlée sur la récupération des plants à la crise de transplantation.

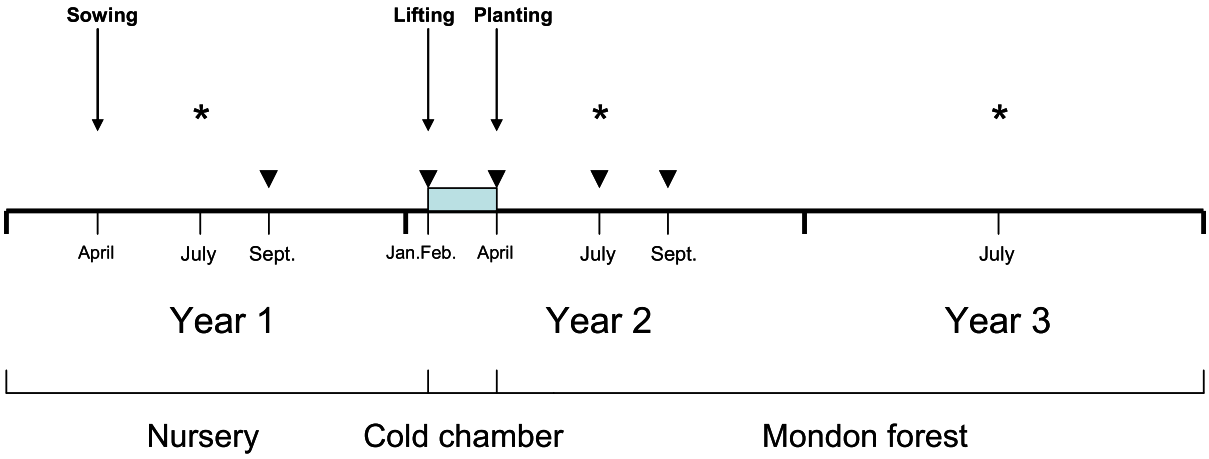


Schéma 2. Diagramme montrant le déroulement de l'expérience. Deux cent plants de *Quercus petraea* ont été semés et inoculés ou non avec *Paxillus involutus* ou *Scleroderma citrinum* puis mis en culture la première année en pépinière à l'INRA (Nancy, France). En début de deuxième année les plants ont été arrachés et stockés deux mois en chambre froide (H.R.: 95%; T: 4°C). Après stockage, les plants on été transplantés en forët de Mondon (Nord Est, France) et suivis pendant deux ans. * : mesures d'échanges gazeux; ▼ : dates d'échantillonnage.

Ce travail a montré le rôle prépondérant des mycorrhizes dans la survie et l'état physiologique des plants à trois ans. Lorsque que les racines étaient ensachées lors du stockage en chambre froide, un rôle très favorisant de la mycorrhization par *Paxillus involutus* a été observé sur le statut hydrique, carboné et azoté des plants. En particulier l'accumulation des réserves carbonées au niveau racinaire avant l'hiver a été stimulée (Figure 5). Il est à noter que 60 à 70% du C, et 40 à 50% de l'N des plants étaient localisés dans le système racinaire en fin de deuxième année. Un article est en préparation sur le sujet [31].

2.6. Dynamiques saisonnières des réserves et flux de C et d'N chez les arbres forestiers adultes

2.6.1. Evolutions saisonnières des réserves C et N chez les chênes pédonculés et sessiles et le hêtre âgés de 50 ans en forêt lorraine

La dynamique saisonnière des réserves carbonées chez des chênes sessile adultes, qui avait été caractérisée de manière très complète par Cécile Barbaroux (2002), a été caractérisée sur ces mêmes arbres pour les composés azotés par Luis Valenzuela Nunes [139]. L'étude sur les réserves protéiques a été réalisée à deux dates (octobre : chute des feuilles et juin : feuilles étalées) et a conduit Valenzuela Nunes à formuler l'hypothèse que la cinétique de stockage des protéines serait décalée dans le temps par rapport à celle des réserves carbonées, le stockage maximum des réserves protéiques se faisant en hiver [18]. Ce travail a aussi permis

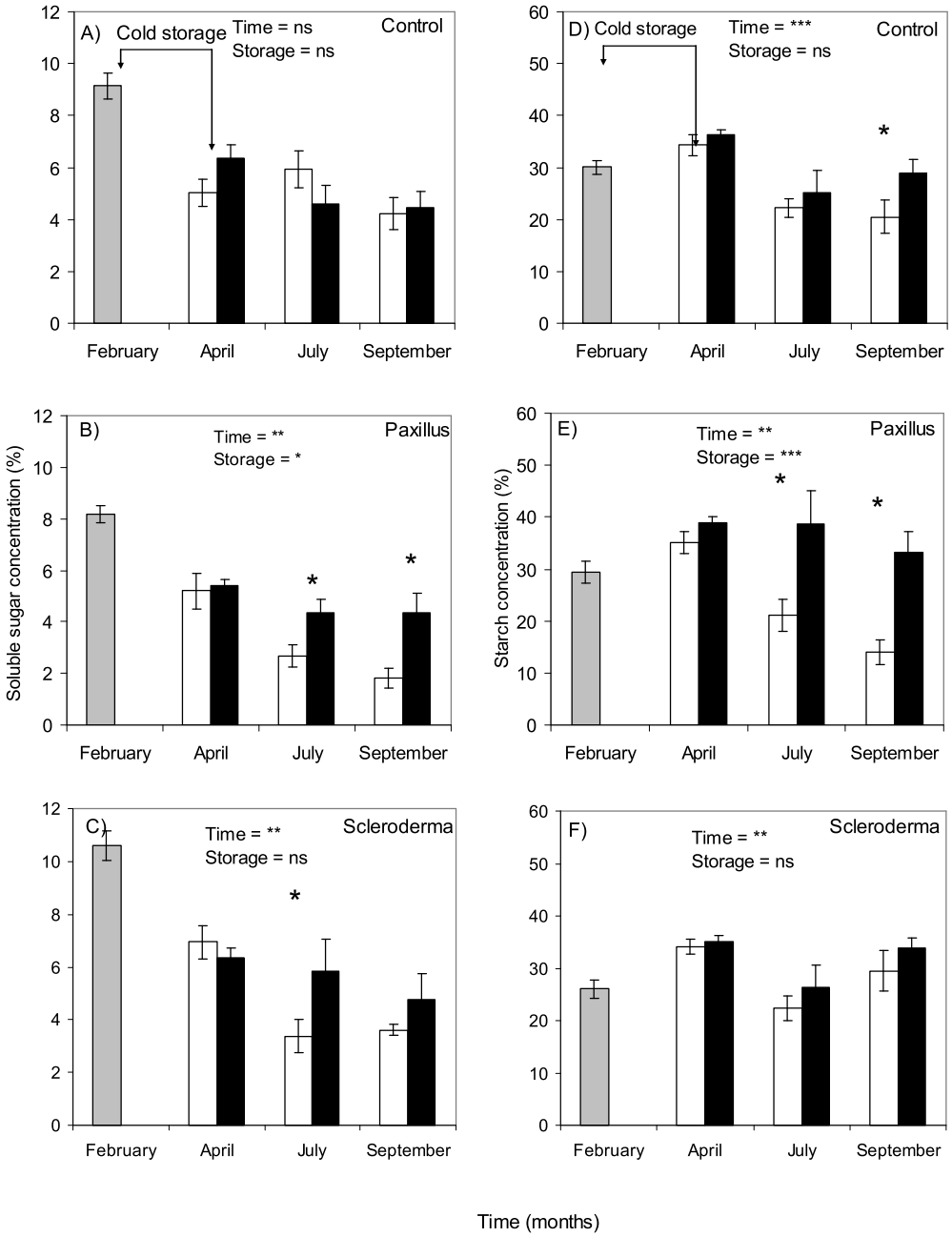


Figure 5. Effet des conditions de stockage au froid avant transplantation en forêt et de la mycorrhization sur les quantités en sucres solubles et en amidon des grosses racines de plants de *Quercus petraea* en fin de deuxième année de croissance. Plants non inoculés (A,D), inoculés au semis avec *Paxillus involutus* (B,E) ou *Scleroderma citrinum* (C,F), durant la première année après transplantation en forêt de Mondon (North East, France).
Gris: Arrachés ; blanc: arrachés et stockés à racines nues; noir: arrachés et racines protégées par un sac.
Analyse de variance à deux facteurs (mode de stockage, temps) ; ns: pas significatif; *, ** et ***: significativement différent à p=0.05, p=0.01 et p=0.001, respectivement (article en préparation).

de montrer que chez les deux espèces de chêne étudiées (Chênes sessile et pédonculé), les réserves protéiques sont dix fois moins importantes que les réserves carbonées. Cependant, l'apport du carbone contenu dans ces protéines (C Protéique), au stock total de carbone à remobiliser au printemps, est non négligeable à l'échelle de l'arbre. Enfin, Luis Valenzuela Nunes a pu mettre en évidence enfin une protéine de stockage (VSP) de 26kDa chez les chênes pédonculé et sessile adultes [19], dont la caractérisation moléculaire est en cours par ma collègue Dominique Gérant.

Grâce à une allocation de recherche (Ecole doctorale RP2E de Nancy-Université) et à un projet émergent de la région Lorraine (j'ai été porteuse à la fois des demandes d'allocation et coordinatrice de projet), ces travaux ont été poursuivis dès la fin 2007 par Rana El Zein (Directeur de thèse N. Breda ; Co- encadrantes : D. Gérant et P. Maillard). Les objectifs de la thèse de Rana El Zein, soutenue début 2011 [115], étaient d'approfondir nos connaissances sur les dynamiques saisonnières des réserves C et N chez le chêne sessile (forêt de Champenoux, Lorraine) et le hêtre (forêt de Hesse, Lorraine) et d'évaluer les périodes critiques au cours desquelles tout événement abiotique ou biotique adverse aura des conséquences néfastes sur la gestion des réserves et donc la pérennité de ces espèces. Un deuxième objectif était la mise au point d'un outil de diagnostic simple de vitalité des arbres en parcelle forestière, qui pourrait prendre à terme la forme d'un kit protéique (travail en cours de D. Gérant).

Un suivi mensuel dans l'aubier de tronc de chênes et de hêtres adultes a été réalisé qui a montré que deux polypeptides de 13 et de 26 kDa s'accumulent uniquement lors de la sénescence foliaire en automne. Alors que les réserves C s'accumulent chaque année dès la fin de l'étalement des feuilles, ces polypeptides très abondants en période hivernale dès que les températures étaient négatives (ce qui est caractéristique d'un endurcissement au froid) étaient totalement indétectables lors du débourrement au printemps. Au printemps, une forte remobilisation des réserves C à partir des cernes les plus récents de l'aubier du tronc a été montrée chez le chêne sessile, mais pas chez le hêtre [6].

2.6.2. Sources d'azote utilisées pour la croissance printanière des branches de chêne sessile

Les contributions respectives de l'N de réserve et de l'N du sol à la construction des nouvelles branches chez le chêne adulte ont été évaluées par Rana El Zein par le biais d'un marquage inédit au ^{15}N de trois chênes sessiles âgés d'une cinquantaine d'années au printemps 2009 (Photo 1).

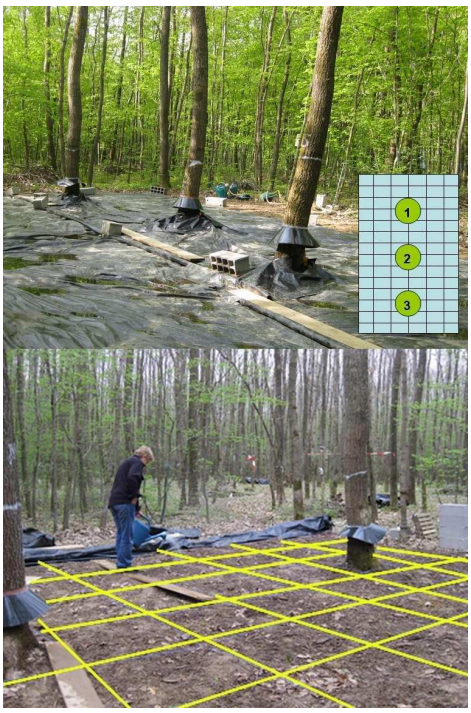


Photo 1. Placette de marquage ^{15}N expérimentale, forêt de Champenoux (France) montrant trois chênes sessiles de 50 ans situés sur une surface de 84 m^2 (14 m * 6 m). Tiré de El Zein *et al.*, 2011 [5].

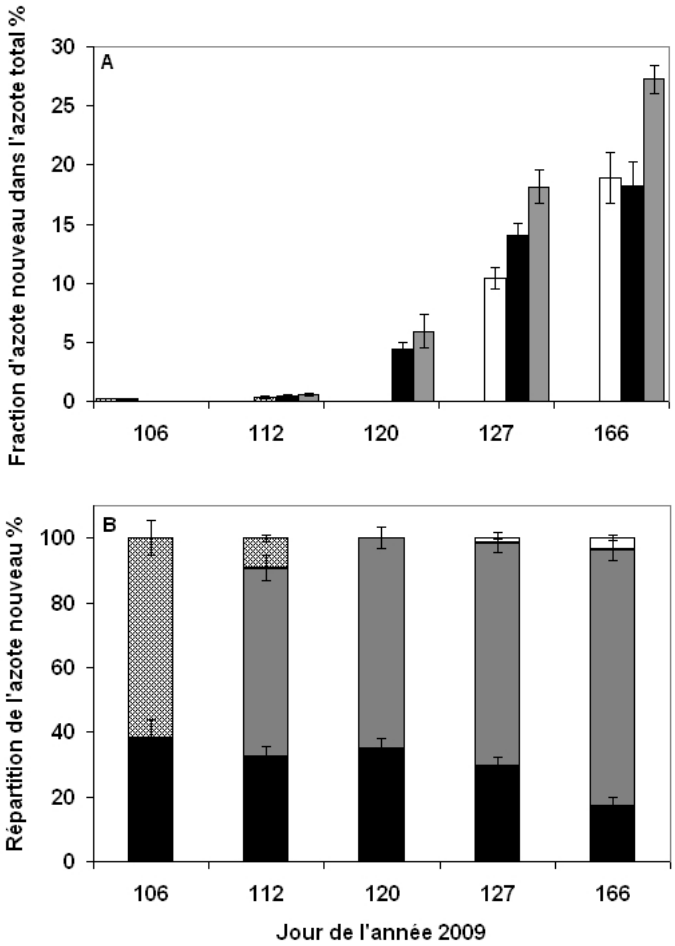


Figure 6. Evolution, du débourrement à l'étalement du feuillage, de la fraction d'azote nouvellement absorbé par l'arbre (chêne sessile âgé de 50 ans) dans l'azote total de chaque organe (A) ainsi que de sa répartition entre chaque organe (B). Branches de 2008 (noir à pois) et 2009 (noir), les bourgeons 2008 (blanc) et 2009 (blanc à pois), et les nouvelles feuilles (gris). Les valeurs obtenues sont la moyenne 6 organes prélevés sur trois arbres ± SE. Tiré de El Zein *et al.*, 2011 [5].

Ce marquage a permis de suivre l'azote issu de l'absorption minérale dans la sève phloèmienne et son incorporation au cours du temps dans les branches en croissance. L'expérience de marquage a permis de montrer que lors de la reprise printanière du chêne sessile adulte, 90% de l'azote (N) des feuilles et des pousses proviennent de l'azote des réserves. L'apparition au débourrement de ^{15}N dans la sève phloémienne et dans les bourgeons suggère que l'absorption minérale est possible à cette période, et qu'en l'absence de feuilles et d'un transport xylémien efficace, l'apport d'N néo-assimilé aux parties aériennes est assuré par le phloème [5]. C'est uniquement à la fin du débourrement complet des arbres que la contribution de l'N néo-assimilé devient importante (Figure 6).

2.6.3. Relations trophiques entre les chênes sessiles adultes et leurs mycorhizes au printemps

La réactivation printanière des deux principales espèces de chêne vivant dans les forêts de l'Europe de l'ouest (*Quercus robur*, *Quercus petraea*) implique l'existence d'une activité cambiale et la formation de bois de printemps avant la formation des nouvelles feuilles (Hinkley et Lassoie, 1981; Bréda et Granier 1996). C'est donc une période critique pour l'arbre car sa croissance dépend uniquement de la mobilisation des réserves carbonées. Barbaroux et Breda (2002) ont démontré l'existence d'une mobilisation importante des sucres non structuraux (sucres solubles, amidon) en réponse aux besoins respiratoires accrus, à la croissance du tronc ainsi qu'au débourrement des bourgeons. Une forte diminution de ces sucres est alors observée dans tous les compartiments de l'arbre. Une disparition du carbone stocké a été quantifiée et comparée au bilan carboné modélisé de l'arbre (Barbaroux, Bréda et Dufrêne, 2003). Le fait que le modèle met en évidence une surestimation de la biomasse de l'aubier et de la respiration et une sous estimation des réserves carbonées disponibles ne permettant pas de boucler le bilan carboné de l'arbre, a été discutée. D'autres sources de carbone ont pu participer à la nouvelle croissance comme le mannose ou des glycoprotéines (El Zein *et al.*, 2011 [6]). Dans les chênes décidus, la réactivation printanière et le débourrement modifient les capacités des ectomycorrhyzes à mobiliser le carbone issu des macromolécules complexes de la matière organique du sol. Courty, Bréda et Garbaye (2007) ont montré que les activités de certaines enzymes cellulolytiques et lignolytiques sont très fortement augmentées chez les ectomycorrhizes *Lactarius quietus* (dominants dans la parcelle forestière étudiée), et qu'elles semblaient corrélées avec la croissance en diamètre de l'arbre et le débourrement des bourgeons. Notre hypothèse de travail a donc été de considérer que peut-être une partie du carbone nécessaire à la réactivation printanière pourrait provenir de la litière, et être transférée temporairement à l'arbre par l'intermédiaire des ectomycorrhizes. Et donc finalement, la question posée était de savoir si le chêne est mycohétérotrophe au

printemps (Projet innovant ; Coordinateurs : Jean Garbaye et P. Maillard). Ce travail a été réalisé en collaboration avec Nathalie Breda et Pierre Emmanuel Courty (post-doctorant).

Afin de mettre en évidence ou non une mobilisation par les chênes d'une source de carbone supplémentaire, d'origine édaphique, au cours de la transition hétérotrophie – autotrophie lors de la réactivation printanière, nous avons utilisé l'approche isotopique et marqué de la litière de peuplier à l'aide de $^{13}CO_2$ (50 atomes %) pendant un mois. Cette litière a ensuite été répartie autour des arbres en hiver sous forme de petits sachets permettant le passage des filaments de mycelium mais pas celui des racines. Une des modalités à consisté en un traitement fongicide appliqué pour réduire la capacité des ectomycorrhizes à mobiliser et transférer les composés marqués au ^{13}C vers l'arbre, et ainsi démontrer par la négative l'importance des mycorrhizes dans ce transfert. Le dispositif expérimental a été mis en place sur le terrain le 17 décembre 2008. Le site choisi était la parcelle 52 de la forêt domaniale de Champenoux, où le peuplement de chêne sessile d'environ 50 ans est déjà instrumenté et intensivement étudié par l'UMR 1137 EEF. Des prélèvements (micorrhizes, racines fines, phloème à 0 et 1,30m de hauteur) ont été réalisés au moment du débourrement en avril, puis en juin. Les résultats isotopiques ont confirmé l'existence d'un flux de carbone marqué du champignon vers l'arbre au printemps. Ce flux de ^{13}C vers l'arbre a été impacté négativement par l'ajout de solution fongicide (Figure 7, une communication orale à un congrès international [47] et un article soumis [26]).

2.6.4. Estimation des flux de C, d'N et d'H_2O entre l'arbre et le sol en fonction des saisons. Mise en relation avec les changements climatiques

La demande politique et sociale sur les estimations des flux et stocks de carbone est actuellement très forte et se manifeste à l'échelle internationale (GIEC). Dans ce cadre, de par l'importance des arbres et des sols forestiers en tant que réservoirs de carbone en interaction avec le CO_2 atmosphérique, les estimations portant sur les écosystèmes forêts sont cruciales. Des modèles sont utilisés pour prédire leurs réponses aux changements climatiques en termes d'échanges de carbone entre biosphère et atmosphère et étudier les boucles de rétrocontrôle entre climat et fonctionnement de l'écosystème. Ces modèles de transferts sol-végétation-atmosphère (SVAT) souffrent d'un manque de connaissance mécaniste sur l'allocation du carbone assimilé et sont de ce fait incapables de prédire précisément le temps de résidence du carbone dans chacun des compartiments de l'écosystème. Par ailleurs, il devient de plus en plus clair que ces modèles doivent permettre d'établir des bilans isotopiques notamment en ^{13}C et ^{12}C. En effet, ceux-ci sont très utiles d'une part, pour déterminer à une plus large échelle la participation des forêts et des continents à l'ensemble des échanges de carbone avec l'atmosphère (utilisation du $^{13}CO_2$ dégagé par respiration) et d'autre part, pour la

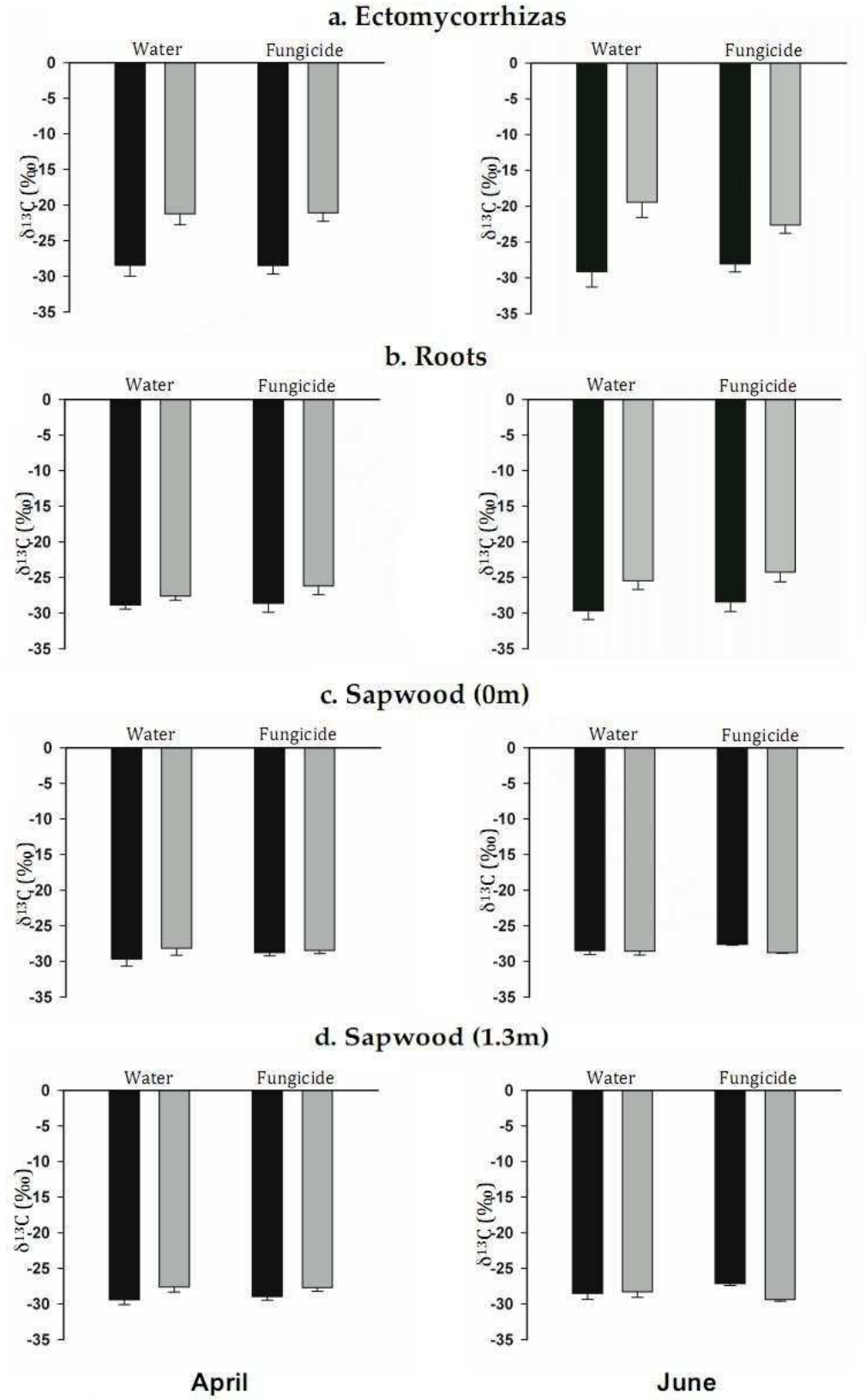


Figure 7. $\delta^{13}C$ de chaque compartiment (mycorhizes, racines, sève phloèmienne prélevée à 0 et 1,30m du tronc) pour chaque date d'échantillonnage (avril et juin). Les sacs contenant la litière non marquée de peuplier (barres noires) ou la litière marquée au ^{13}C (barres grises) ont reçu soit de l'eau soit un traitement fongicide. Article soumis [26].

reconstitution des paléoclimats du Quaternaire (utilisation du ^{13}C des cernes). Le projet CATS (Integrated Monitoring of Carbon Allocation in Tree and Soil, ANR 2007 « CATS », coordinateur : Pr. D. Epron) proposait de combler ces manques par une meilleure caractérisation et compréhension de l'allocation du carbone dans les forêts en termes de carbone total et de ses formes isotopiques ^{12}C et ^{13}C. Le projet CATS a reposé sur une nouvelle technologie (TDL, spectrométrie à diode laser) permettant de mesurer *in situ* à haute fréquence les teneurs en $^{13}CO_2$ et $^{12}CO_2$ et dont le gros équipement a été récemment acquis par les trois laboratoires partenaires du projet.

Les ambitions du projet résidaient (1) dans l'ampleur des expérimentations proposées in situ, (2) dans le développement d'une vision intégrative du fonctionnement carboné des forêts en prenant en compte simultanément arbre et sol et ce jusqu'à des compartiments biochimiques fins, et (3) dans un cheminement complet depuis l'expérimentation jusqu'à la modélisation, et ceci grâce aux compétences et complémentarités des équipes. Plus précisément les objectifs ont été (1) de suivre et de quantifier le ^{13}C dans les organes de l'arbre, la biomasse microbienne et les flux respiratoires au dessus et au dessous du sol après un marquage court réalisé in situ à différentes dates durant la saison de végétation croissance différentes, (2) de mieux décrire le fractionnement isotopique durant la respiration, (3) de résoudre temporellement la déposition radiale du ^{13}C dans le cerne en croissance et d'estimer la mobilisation des réserves pour la formation du nouveau cerne, et (4) d'améliorer nos modèles SVAT en les rendant capable de simuler correctement la signature isotopique du CO2 respiré et des composants structuraux du bois.

La TDL et les isotopes stables ont été utilisés en conditions naturelles pour étudier la vitesse de transfert du carbone depuis l'arbre vers le sol, identifier les différentes sources de carbone de CO_2 respiré, et pour caractériser la maturation des cernes. Des expérimentations lourdes de marquage *in situ* d'arbres (Figure 8), ont permis le suivi du carbone dans les différentes molécules de l'arbre (structure, carbone labile ou de réserve, carbone respiré) et du sol (microorganismes, carbone respiré) pendant plusieurs jours à plusieurs mois suivant le marquage et ainsi de préciser spatialement et temporellement les schémas d'allocation du carbone assimilé. Le travail a été réalisé sur trois sites équipés de tour à flux (Hesse, Bordeaux, Barbeaux) et a porté sur trois espèces forestières (Chêne, Hêtre, Pin maritime) largement représentées dans les forêts françaises et caractérisées par des phénologies variées [2].

Une grande chambre de marquage (arbres de plus de 10m de haut) mobile a été conçue, qui a permis d'enfermer la couronne d'un arbre (Figure 8). Il a nécessité des mesures continues de la composition isotopique du CO_2 des flux respiratoires, et il a impliqué

également de suivre le carbone dans les métabolites, dans les composés de réserve et de structure, et dans les microorganismes du sol. Comprendre et prédire la signature isotopique du CO_2 respiré et du cerne nécessitaient une meilleure caractérisation du fractionnement biochimique et diffusif qui s'opère le long du trajet du carbone dans la plante et le sol. Les résultats du projet sont maintenant disponibles pour améliorer les schémas d'allocation du carbone et pour inclure le fractionnement isotopique des processus clefs.

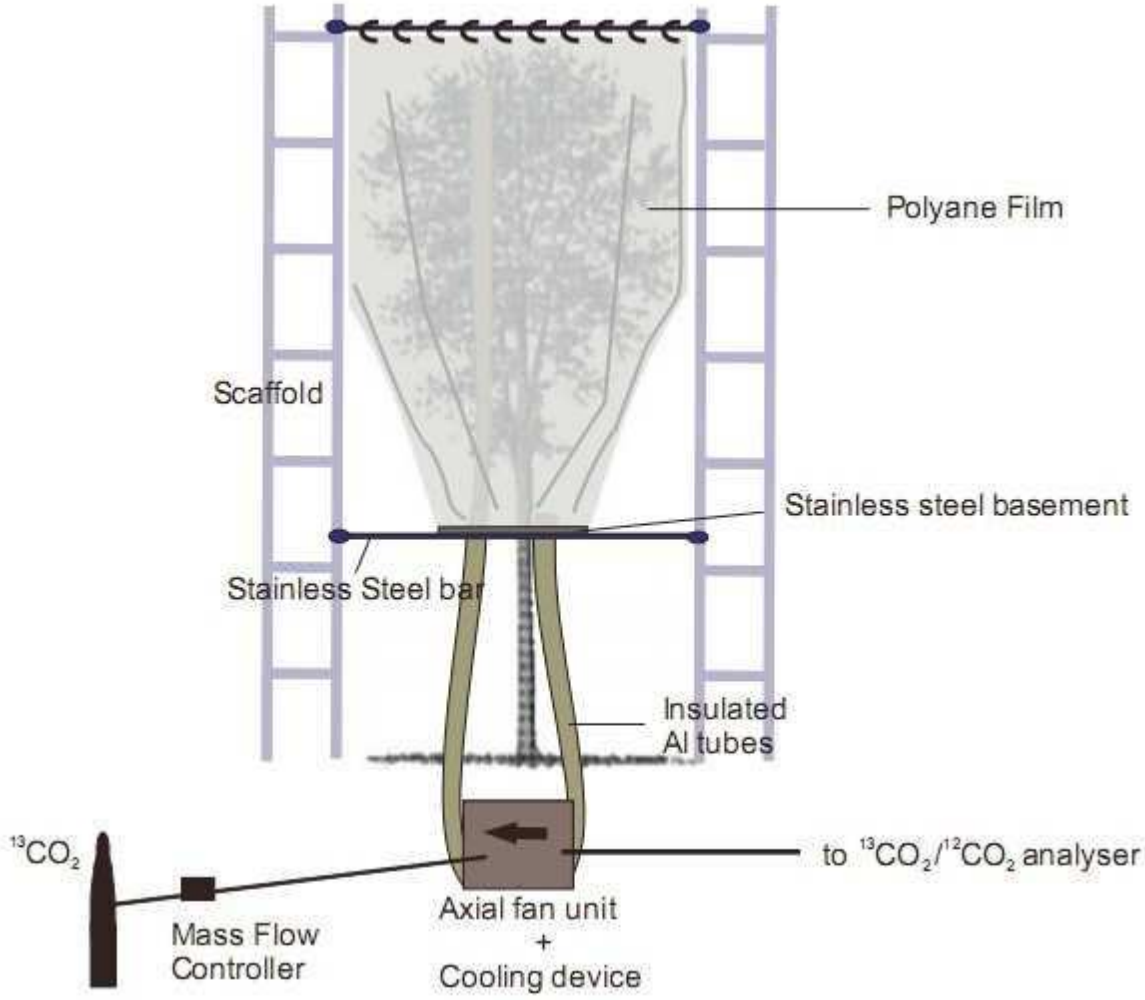


Figure 8. Schéma de la chambre de marquage. La concentration en $^{13}CO_2$ et $^{12}CO_2$ était mesurée en continu au niveau de l'entrée du système de refroidissement. L'injection de $^{13}CO_2$ a été réalisée au niveau de la sortie de ce système. Tiré de Plain *et al.*, 2009 [22].

J'ai participé activement à ce projet qui a impliqué de nombreux partenaires INRA et universitaires et des étudiants en master. Les premières expérimentations ont débuté en septembre 2008 et se sont poursuivis jusque fin 2010. Ce projet avait pour objectif une meilleure caractérisation de l'allocation du carbone dans l'arbre et dans le sol (carbone total, ^{13}C et ^{12}C) en situation naturelle (hêtraie, Hesse), avant l'hiver (2008), au printemps (2009), et après un stress hydrique estival (expérimentation 2009). Des expérimentations de marquage d'arbres durant la saison de végétation ont permis le suivi du carbone de l'arbre (C structural,

C labile ou de réserve, C respiré) et du sol (C structural, C labile des microorganismes, C respiré) pendant plusieurs jours à plusieurs mois suivant la durée du marquage. Ce projet à la fois très enthousiasmant humainement (équipe soudée, nombreux étudiants) et scientifiquement parlants a nécessité l'utilisation de nombreuses techniques physiques et biochimiques afin de caractériser le cycle du carbone arbre-sol-atmosphère d'une jeune hêtraie. Le projet reposait sur la conception d'une chambre de marquage permettant d'insérer la totalité de la couronne d'un arbre, et sur une nouvelle technologie de spectrométrie d'absorption à diode laser modulée permettant de mesurer in situ à haute fréquence les concentrations en $^{13}CO_2$ et $^{12}CO_2$ [22]. Cette approche a permis de mesurer l'évolution de la composition isotopique des flux respiratoires, et ainsi de déterminer la vitesse de transfert du C dans le tronc (Figure 9).

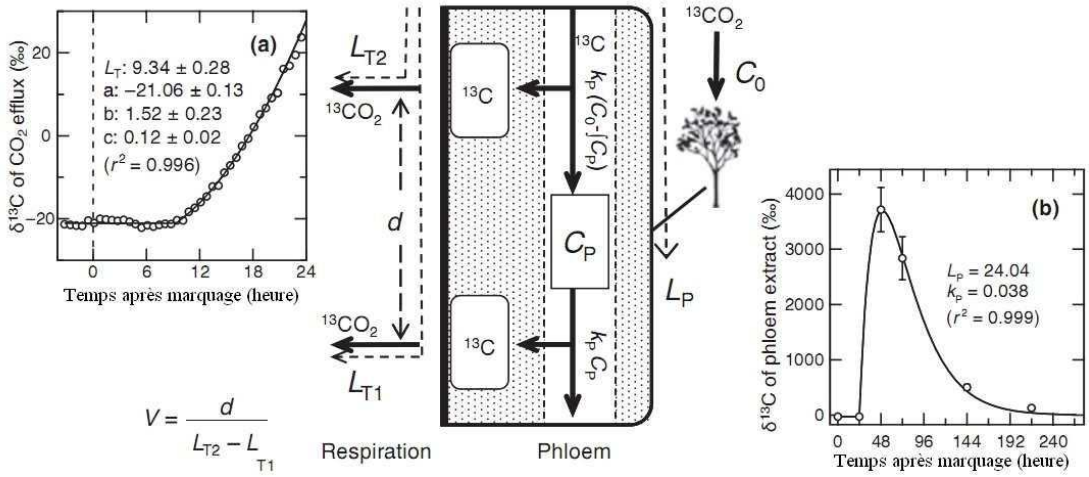


Figure 9. Représentation schématique montrant la respiration à deux niveaux dans le tronc de l'arbre sur la gauche et le flux de carbone marqué dans le phloème sur la droite. (a) Détermination du temps de latence (LT) entre le début du marquage et l'apparition de ^{13}C dans l'efflux de CO_2 par ajustement à l'aide d'une fonction quadratique de la relation entre δ13C et le temps depuis le début du marquage (Eqn 2). Exemple chez le pin maritime en août 2009. Tiré de Dannoura *et al.*, 2011 [2].

Celle-ci est très variable entre les deux feuillus (0,2 à 1,2 m h^{-1}) et le pin (0,1 à 0,2 m h^{-1}) [2]. De ce fait, le temps de latence entre le marquage et l'observation d'un excès de ^{13}C dans la respiration du sol est de 10 à 32 h chez le hêtre et le chêne, et de 2 à 5 jours chez le pin [43, 44]. La quantité totale de ^{13}C allouée à la respiration du sol est très variable selon les saisons chez le hêtre (jusqu'à 20% en juillet lorsque la croissance aérienne est terminée) mais beaucoup moins variable chez le chêne (env. 10%). Elle varie également beaucoup entre la saison de végétation et l'hiver chez le pin (entre 10% et 1%). Du ^{13}C est incorporé dans la cellulose du bois plusieurs semaines après la fin de l'élongation cellulaire dans le tronc, et est mis en réserve (C non structural) dans les organes pérennes des arbres en fin de saison végétative.

3. Perspectives scientifiques

La connaissance des processus d'allocation des ressources entre les différents organes et les différentes fonctions de l'arbre est encore trop parcellaire pour pouvoir être intégrée de manière satisfaisante, dans des modèles mécanistes de fonctionnement de l'écosystème forestier. Notre activité s'est principalement focalisée sur deux compartiments (1) les réserves carbonées (C) et azotées (N) dont le rôle est crucial chez les espèces pérennes décidues et (2) le compartiment souterrain qui reçoit une fraction importante de la production primaire brute mais qui est mal caractérisé, à la fois en terme de composante (racines, rhizosphère, matière organique) et de temps de résidence.

Les recherches de mon équipe (Arbeco) s'appuient fortement, dans plusieurs de nos opérations de recherche, sur le site-atelier de Hesse (réseaux ORE F-ORE-T, Carboeurope, et Fluxnet), une jeune hêtraie de plaine et une jeune fûtaie de chêne, représentatives de larges surfaces forestières semi-intensives en France et en Europe de l'ouest. Toutefois, dans le prochain contrat quadriennal, notre projet prendra en compte de nouveaux objets, en raison de leur importance environnementale ou économique, mais aussi pour être en mesure de comparer leurs propriétés respectives.

Une réflexion menée à l'INRA (CARBIO1) sur l'utilisation de la biomasse végétale à des fins énergétiques a conduit à relancer les recherches sur les taillis à courte révolution (TCR). Notre compétence sera mise au service de la quantification du bilan écologique de ces plantations, en termes d'efficience d'utilisation de l'eau et de l'azote et de maintien de la fertilité, en référence aux peuplements forestiers classiques que nous étudions. Ce programme a démarré dès 2008 avec le recrutement de Nicolas Marron (CR1, Equipe Arbeco), et nous avons des collaborations en cours avec le CIRAD sur les plantations tropicales d'Eucalyptus (Projet Intens&Fix , 2011-2015).

3.1. Estimation des flux de C, d'N et d'H_2O en forêt

3.1.1. Suivi à haute résolution (spatiale et temporaire) de l'allocation du carbone et de l'azote à l'échelle arbre - sol d'une hêtraie lorraine par triple marquage isotopique (^{13}C, ^{15}N, ^{2}H)

Nous savons que l'allocation du carbone assimilé entre les compartiments d'un arbre (houppier, tronc, système racinaire) est contrôlée par l'environnement et qu'elle affecte la croissance des arbres, la contribution de chacun des organes de la respiration autotrophe, le transfert de carbone dans la rhizosphère et, *in fine*, la séquestration de carbone dans l'écosystème (Magnani *et al.*, 2002 ; Giardina *et al.*, 2003). Des études menées notamment sur des espèces herbacées d'intérêt agricole (Morot-Gaudry et Lea, 2001) et de jeunes chênes et hêtres cultivés en conditions semi-contrôlées (Valenzuela-Nunes, 2006 ; Vizoso *et al.*, 2008)

ont démontré qu'une des composantes de l'environnement, l'azote du sol, est un élément clé qui pilote la croissance et interagit donc de manière étroite avec l'allocation du carbone.

La partie aérienne des arbres (feuilles, branches, tiges) contribue à la respiration de l'écosystème, mais les deux tiers des sources de C sont situés dans le sol (Janssens *et al.*, 2001). Le sol inclue bien sûr les racines, mais aussi des organismes symbiotiques : microbes de la rhizosphère qui se nourrissent des exsudats racinaires (sucres, acides aminés, …), bactéries et champignons saprophytes qui se nourrissent de débris ligneux grossiers, de litière hors-sol et de litière souterraine (Raich et Schlesinger, 1992; Nadelhoffer et Raich, 1992; Epron *et al.*, 2001; Ngao *et al.*, 2005). Bien que l'importance des flux de carbone des racines vers la rhizosphère et les mycorrhizes ait été reconnue pour les écosystèmes forestiers, celle-ci est mal documentée par des mesures *in situ* (Högberg et Read, 2006). L'importance des flux d'azote des systèmes racinaires vers la rhizosphère est encore très mal estimée encore en milieu forestier tempéré (Högberg, 1997). Pourtant, la compréhension du cycle de l'azote dans les écosystèmes forestiers est essentielle notamment du fait d'une augmentation des dépôts azotés dans de nombreuses régions de l'Europe.

Dans le cadre du projet innovant que nous avons présenté au département EFPA, nous nous sommes proposés d'étudier sur des hêtres adultes en forêt de Hesse (site atelier, Lorraine, France) les flux d'azote (marquage ^{15}N) et de carbone (marquage ^{13}C) internes à l'arbre, et ceux en direction de la rhizosphère et des populations microbiennes. Par le biais d'un triple marquage (^{13}C, ^{15}N, ^{2}H). Ce faisant, nous nous attacherons à suivre les dynamiques d'allocation du carbone et de l'azote dans l'arbre (stockage hivernal) en fin de saison de végétation et jusqu'au printemps suivant (nouvelle croissance) ainsi que les relations trophiques dynamiques qui existent entre l'arbre et le sol. Une telle étude, permettra d'apporter des informations mécanistes originales et innovantes sur les temps de résidence du C et de l'N dans le système arbre-sol d'un écosystème forestier tempéré, durant l'inter saison hiver printemps. Enfin, le marquage ^{2}H (pulvérisation foliaire), nous permettra de tester l'hypothèse selon laquelle l'information apportée par le marquage de l'eau foliaire apporte des informations analogues à celles apportées par un marquage du CO_2 (beaucoup plus complexe à mettre en place en forêt et sur arbres adultes) les éléments C et H s'intégrant de façon similaire dans les composés (Pataki *et al.*, 2003, E. Deléens, communication personnelle).

Ce projet constitue une suite du projet CATS et Bernd Zeller (Unité BEF) et moi-même avons bénéficié de l'infrastructure et des moyens développés par le projet CATS (échafaudages en forêt, mesures de flux de C en continu, moyens humains) pour réaliser en automne 2008 un double marquage ^{15}N et ^{2}H (pulvérisation foliaire) sur deux hêtres

simultanément marqués au ^{13}C en site atelier de la forêt de Hesse. Le triple marquage (^{13}C, ^{15}N, ^{2}H), les campagnes d'échantillonnage sol-arbre et les analyses ^{13}C de la biomasse et de l'air respiré du sol et des arbres ont été pris en charge par le projet CATS.

En revanche, la compréhension et l'établissement d'un bilan des flux d'azote dans les hêtres et le sol et leur interaction avec les flux de carbone qui constitue le corps et l'originalité de notre projet innovant, impliquait des analyses isotopiques ^{15}N et ^{2}H, de l'eau du sol, des populations microbiennes du sol, et des arbres (tronc, branches, feuilles, racines) non prévues à l'origine dans le projet CATS.

Notre projet, en réunissant une équipe interdisciplinaire comprenant à la fois des chercheurs des sciences du sol (UMR 1138, D'Annunzio *et al.*, 2008) et de l'écophysiologie de l'arbre (UMR 1137, Vizoso *et al.*, 2008) répond à un objectif commun qui est de mieux comprendre les cycles du carbone et de l'azote à l'échelle de plusieurs saisons dans l'arbre et entre l'arbre et le sol. Les approches visent à intégrer des fonctionnements cellulaires à l'écologie d'espèces forestières d'intérêt, et à accéder à des dynamiques intra et inter annuelles par le biais d'outils isotopiques et biochimiques. Une des originalités du projet est enfin de réaliser cette étude sur des arbres adultes d'une placette dont toutes les caractéristiques édaphiques et atmosphériques sont suivies (site atelier de Hesse).

Un étudiant en master 1 FAGE (Janick Moukambo) que j'ai encadré en 2011 a démarré l'étude de l'incorporation du ^{15}N (marqueur des réserves azotées) dans les pousses de hêtre (bourgeons, tige, feuilles) des arbres marqués. L'objectif de son stage était de suivre par marquage ^{15}N, l'azote foliaire exporté en automne, avant la chute des feuilles, vers les compartiments pérennes de l'arbre, et son devenir au printemps, sur deux hêtres de 15 ans d'une hêtraie lorraine. Cet étudiant s'est plus particulièrement intéressé au stockage et à la remobilisation de l'azote dans les branches et au suivi de son utilisation au printemps par les nouveaux organes (tiges, feuilles) : Les analyses isotopiques ont été réalisées sur les branches et les nouvelles pousses prélevées régulièrement, de la date de marquage (Septembre) jusqu'au printemps suivant à l'étalement complet des nouvelles feuilles.

Les premiers résultats suggèrent que les branches 2008 et les bourgeons stockent davantage l'azote foliaire issu de la remobilisation foliaire automnale, que les grosses racines (non montré). L'hypothèse à vérifier serait que l'azote foliaire est stocké à proximité des feuilles de l'année 2008 avant leur sénescence, c'est-à-dire dans les branches et les bourgeons, ce qui rendrait cet azote disponible rapidement au printemps suivant pour les organes puits en croissance telles que les tiges et les feuilles. Les résultats montrent aussi que la teneur en ^{15}N

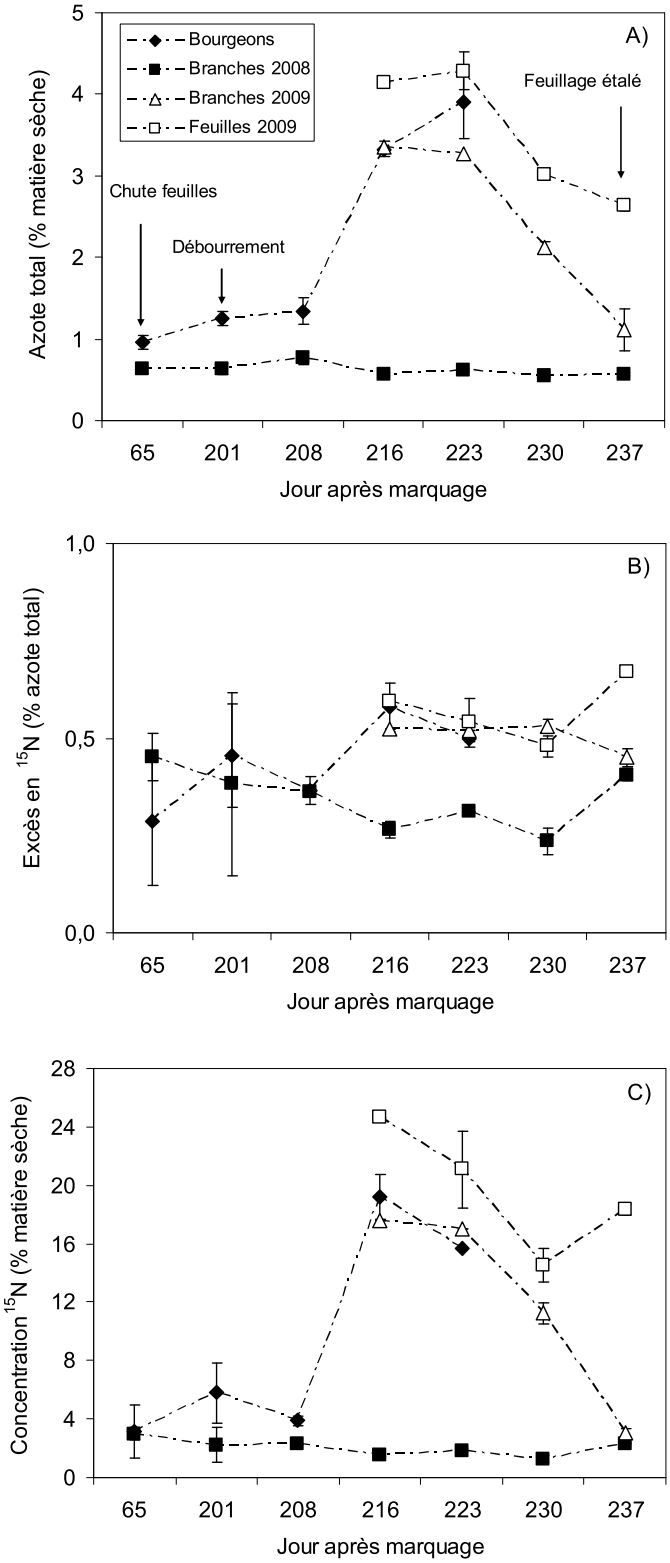


Figure 10. Evolution au cours du temps de la teneur en azote total (A), de l'excès isotopique (B) et de la concentration en ^{15}N (C) des organes aériens pérennes (bourgeons, branches 2008) et des nouveaux organes (branches 2009, feuilles 2009) de hêtres âgés de 15 ans en forêt lorraine (Hesse, France). Echantillons prélevés à l'automne après la chute des feuilles (jour 65), et une fois par semaine du débourrement (jour 201) à l'étalement complet du nouveau feuillage (jour 237). Chaque valeur est la moyenne ± erreur standard (n = 2 à 5). Pour les branches 2009, les jours 216 et 223 n=1.

des branches pérennes diminue alors que celles des pousses en croissance augmente (Figure 10). Les nouveaux organes ont une teneur en azote plus élevée que celles des organes pérennes, et sont des puits importants pour l'azote. De plus, le marquage ^{15}N montre que les nouveaux organes se concentrent beaucoup plus fortement en ^{15}N que les organes pérennes ce qui démontre que ces organes utilisent prioritairement les réserves azotées pour assurer leur développement (Figure 10C). Ces résultats sur hêtre rejoignent donc ceux que nous avons obtenus sur chêne [5], à savoir que les nouvelles branches utilisent majoritairement les réserves azotées pour leur croissance au printemps.

Ce travail d'analyse est préliminaire et sera poursuivi. Les données N acquises sur l'arbre seront confrontées à celles du sol (UR BEF, Bernd Zeller) afin de boucler le bilan arbre-sol pour l'azote. La comparaison des deux marquages ^{13}C et ^{2}H et des informations apportées par chacun des deux éléments sera réalisée afin de valider ou invalider l'hypothèse selon laquelle le marquage de l'eau foliaire apporte des informations analogues à celles apportées par un marquage du CO_2 (beaucoup plus complexe à mettre en place en forêt et sur arbres adultes).

3.1.2. Importance des réserves carbonées et azotées dans l'allocation du C et de l'N à la croissance et au stockage d'espèces pérennes en réponse à la défoliation et aux sècheresses estivales

A la suite de ce travail, qui nous apporte des informations fondamentales sur la gestion du C et de l'N sur arbre adulte en situation naturelle favorable, j'envisage de monter un projet type ANR blanc avec mes collègues, dont l'objectif sera de comprendre l'importance que revêtent les réserves C et N dans l'acclimatation aux stress biotiques et abiotiques. Les stress retenus sont la défoliation et les sècheresses estivales susceptibles de prendre de l'ampleur du fait du réchauffement climatique en cours. Notre équipe est en phase de réflexion sur ce projet afin d'y associer nos collègues CNRS d'Orsay (arbres forestiers), et INRA de Clermont-Ferrand (arbres forestiers, plantes herbacées), d'Avignon (arbres fruitiers), et de Caen (plantes herbacées), préoccupés par les mêmes questions mais sur des modèles végétaux et des attendus de production différents.

Ce thème qui est en phase avec le thème transversal que notre UMR souhaite développer dès 2013, sera l'occasion d'aborder un certain nombre de questions sur arbres adultes, auxquelles nos études sur jeunes plants n'ont pas encore permis d'apporter de réponse. En particulier nous ne savons pas bien si c'est le niveau de réserves carbonées ou celui des réserves azotées qui limite la croissance en situation de stress. Les informations disponibles dans la littérature et en particulier celles d'auteurs comme Hoch *et al.* (2003) et

Millard *et al.* (2007) montrent qu'il y a rarement épuisement des réserves carbonées dans les arbres. En revanche, concernant les réserves azotées une diminution drastique des réserves azotées, notamment pour assurer la croissance printanière, peut se produire dans les organes pérennes (Millard, 1996) en particulier en situation de carence en azote ([141] ; Vizoso *et al.*, 2008). Un déficit de mise en réserves C peut se traduire par une surface foliaire réduite l'année suivante entraînant l'arbre dans un cercle vicieux qui peut s'avérer fatal en cas d'attaque parasitaire chez le chêne en forêt (Marçais et Breda, 2006). En revanche, concernant les réserves azotées nous n'avons pas encore d'informations claires. Cependant, sur jeunes plants nous avons pu constater que les réserves azotées ne s'épuisaient pas après défoliation, ceci grâce à une compensation des pertes en azote par l'absorption minérale et à une diminution de la croissance. Les plants de notre expérience étaient fertilisés et aucun stress hydrique n'était appliqué. En situation de sècheresse et sur sol forestier pauvre il est probable que la réponse des arbres soit différente.

Une deuxième question à laquelle nous aimerions apporter des réponses sur arbre adulte dans notre projet, est de tester l'hypothèse émise pour le C par Davi *et al.* (2009) selon laquelle le compartiment réserves est un puits actif qui peut devenir prioritaire au détriment de la croissance en cas de stress important aboutissant à une diminution drastique des réserves dans l'arbre.

Enfin, nos travaux sur les branches de hêtre et de chêne nous amènent à penser que les branches, qui représentent un compartiment de stockage quantitativement important dans l'arbre (Gérant *el al* ; soumis), sont des organes de stockage majeurs et de proximité dans l'approvisionnement en C et en N du nouveau feuillage. Aussi, nous comptons porter un intérêt particulier à ces organes dans le projet à venir, d'autant plus que leur autonomie trophique (Lacointe *et al*., 2004) peut-être altérée par les défoliations et toucher différemment les branches d'ombre et de lumière.

3.1.3. Comparaison des flux C-H_2O saisonniers de deux hêtraies européennes (Lorraine et Bade Wurtemberg)

Dans le cadre de ce projet (Projet CarboEurope, responsable André Granier) en partenariat avec une équipe allemande dirigée par Arthur Gessler [A. Gessler Professor of Landscape Biogeochemistry, Institute for LandscapeBiogeochemistry Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF) Eberswalderstr. 84, 15374 Müncheberg], j'ai participé depuis l'année 2007 (mars à octobre) à une expérience visant à caractériser de manière bimensuelle les flux C-H_2O arbre-sol-atmosphère et à comparer leurs variations saisonnières dans deux hêtraies jeunes (Sites ateliers de Hesse et de Tuttligen). Ce travail utilise des approches isotopiques (^{18}O, ^{13}C). Ce travail a généré de nombreux échantillons (France,

Allemagne), dont l'extraction puis l'analyse isotopique (eau du sol et de l'air, feuilles et branches, sève phloèmienne) viennent de s'achever. Ce travail qui m'a permis d'apprendre la technique d'extraction de sève phloèmienne mise au point par A. Gessler, et de l'utiliser dans d'autres projets en cours, devrait après discussion avec A. Gessler permettre la comparaison du fonctionnement de deux hêtraies européennes et déboucher sur une publication à comité de lecture.

3.2. Etude de la gestion des réserves carbonées et azotées de plantations de peuplier et de robinier à courte rotation

À l'heure où la recherche de solutions alternatives à l'utilisation des carburants fossiles devient un sujet prépondérant pour la communauté scientifique mondiale, la biomasse végétale pourrait représenter une option écologiquement et économiquement satisfaisante. Parmi les nombreuses sources de biomasse, la biomasse ligneuse a l'avantage de séquestrer le carbone pour des périodes plus longues et de présenter un bilan environnemental plus favorable que les espèces annuelles. La production de ce type de biomasse se fait généralement par l'intermédiaire de plantations à rotations courtes ou très courtes (souvent 2 à 7 ans ; Figure 11) composées d'espèces à croissance très rapide comme le peuplier, le saule ou le robinier.

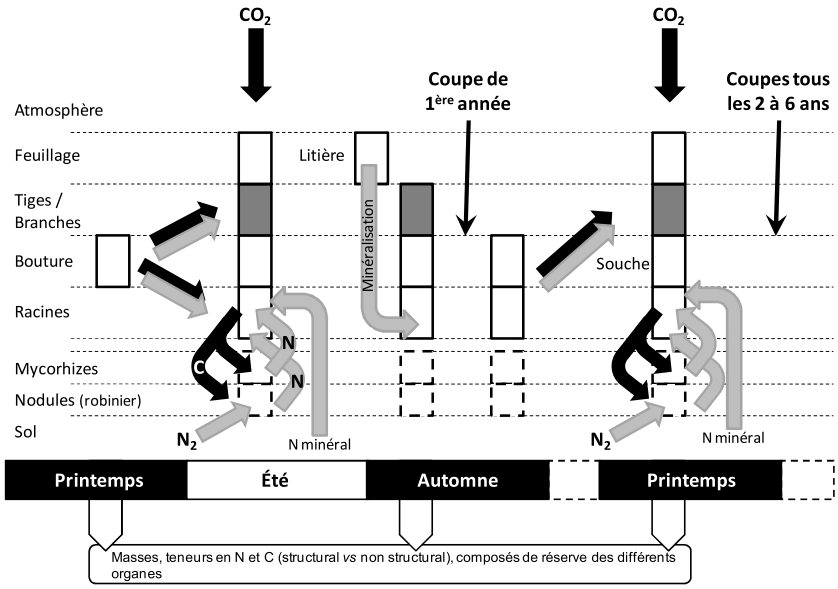


Figure 11. Représentation schématique de la dynamique interannuelle des flux de carbone (flèches noires) et d'azote (flèches grises) au sein d'un taillis à courte rotation (TCR).

Dans ce contexte, les objectifs du présent projet [(2008-2015 Projet émergent région lorraine + Projet ANR Intens & Fix)] sont de comparer les métabolismes carboné et azoté et leurs dynamiques aux échelles saisonnière et interannuelle (intra- et inter-rotations) chez deux genres contrastés mais bien adaptés à la culture à courte rotation, le peuplier et le robinier, ce dernier présentant la propriété des légumineuses de fixer l'azote atmosphérique. Deux

attendus sont la mise en évidence un itinéraire technique optimal et des outils diagnostiques de prédiction de l'épuisement du système (peuplier robinier) et des besoins subséquents de fertilisation. La réalisation du dispositif de culture est faite et les expérimentations commenceront cette année 2012 à Saint Cyr en Val près d'Orléans. Il est à noter que ce projet est d'envergure internationale puisqu'il implique dans le cadre de l'ANR Intens&Fix (coordination : J.P. Bouillet) financé jusqu'en 2015, des partenaires français (CNRS, CIRAD) mais aussi bréziliens (projet de coopération bilatérale, Université de Sao Paulo University, USP-COFECUB project "Introducing nitrogen fixing species in forest plantation: effects on growth and processes").

Les études sur l'association peuplier-robinier seront comparées avec celles menées sur des cultures d'eucalyptus pures ou en mélange avec des espèces ligneuses fixatrices d'azote (Acacias) au Brésil et au Congo.

Je participerai à hauteur de 30% de mon temps chercheur à ce projet. Ce sera aussi l'occasion pour moi d'aider au travail de thèse Morgane Pluchon (Direction D. Epron ; co-encadrement D. Gérant) qui démarre sur le sujet, à la fois en tant que membre de son comité de thèse mais aussi pour abonder les aspects isotopiques et de gestion du C et de l'N.

Plus généralement, les compétences en écophysiologie, physiologie et biochimie présentes au sein de notre équipe seront mises à contribution. L'allocation et la dynamique des pools d'azote et de carbone seront évaluées par l'intermédiaire de mesures régulières de la croissance aérienne et racinaire des deux espèces, des échanges gazeux, et d'un marquage ^{15}N du sol. Des plants de robinier seront également marqués par le biais d'un injection de ^{15}N dans la sève xylémienne (technique UR BEF, Bernd Zeller). Ces marquages et le suivi du ^{15}N dans les arbres devraient permettre de déterminer le stock d'azote annuel des arbres et d'estimer l'effet fertilisant du robinier pour le sol et le peuplier. Les modalités de marquage encore en discussion, seront mises en œuvre à l'été 2012.

3.3. Traçage et modélisation des flux de carbone chez l'hévéa, de l'assimilation au latex (projet Fluxomic soumis)

Les flux de C sont généralement évalués indirectement par les variations de taille ou d'activité des organes puits (comme la croissance du fruit). Ces flux estimés peuvent cependant être biaisés à une échelle de temps donné, notamment dans le cas de réserves dont le stock peut paraître inchangé alors qu'il est la somme de phénomènes de mobilisation et de restockage sous-jacents. Les flux en provenance des organes source de C vers les organes puits peuvent être mesurés directement grâce à l'utilisation de composés marqués. L'utilisation de $^{13}CO_2$ permet ainsi de suivre le carbone assimilé et son transfert à travers le phloème du feuillage vers les organes puits. Cette méthode longtemps utilisée sur des plants de petite

taille et en laboratoire, a récemment été étendue à des arbres de grande dimension, fournissant des informations importantes sur les processus d'allocation du C en forêt ([2], Epron *et al.*, 2011). Cependant, ces études n'ont pas encore abordé sur arbre adulte la question de l'influence des variations d'activité des puits de C sur l'assimilation photosynthétique et les schémas de répartition des photoassimilats. Que ce passe-t'il si un puits supplémentaire entre compétition pour le C avec les puits existants ? L'arbre à caoutchouc (*Hevea brasiliensis*) et la production de latex est un arbre modèle très pertinent pour étudier cette question. Lorsque les vaisseaux situés sous l'écorce sont coupés par la pratique de la saignée, le latex riche en C s'écoule et est recueilli avant d'être régénérés *in situ*. Cette pratique induit un puits artificiel de C qui peut jouer sur la dynamique des réserves carbonées (Silpi *et al.*, 2007).

L'un des objectifs du projet Fluxomic Hevea est d'étudier l'effet de la saignée sur l'assimilation du C et sur son transport dans l'arbre. À cette fin, une expérience de terrain sera effectuée en Thaïlande. Des hévéas en plantation seront marqués au $^{13}CO_2$ (marquage court et fort de la couronne) en utilisant des modalités proches de celles du projet CATS. Le marqueur sera suivi de son assimilation dans le feuillage à son arrivée dans les différents compartiments de l'arbre (tronc, racines). Son incorporation dans les composés métaboliques soluble à fort turn-over et insolubles de réserve ainsi que dans le latex produit sera recherchée. Les résultats seront synthétisés dans les modèles d'arbres fonctionnels en tenant compte du transfert observé C et les modèles de répartition. En plus de la connaissance générique en écophysiologie des arbres, ce projet apportera des informations sur l'origine des substrats carbonés utilisés (assimilats courants, réserves) pour la synthèse de latex C. Cela permettra d'améliorer la compréhension du métabolisme du latex dans l'arbre, et contribuera également à apporter les clés d'une meilleure gestion des systèmes d'exploitation de l'hévéa.

Daniel Epron (Leader tache 5) et moi-même travaillerons sur la tache 5 du projet. Les objectifs de cette tâche seront d'étudier les dynamiques de transfert du carbone dans l'arbre. Après marquage au ^{13}C de l'arbre nous collecterons la sève phloémienne à deux hauteurs le long du tronc ainsi que des feuilles de lumière. Nous analyserons la composition biochimique des composés structuraux et non structuraux de ces compartiments et déterminerons leur enrichissement en ^{13}C. Les méthodes de suivi du dépôt du carbone marqué dans les structures sera à préciser, car les cernes de croissance ne sont pas toujours nets sur les espèces tropicales. Le marquage nous permettra d'évaluer le temps mis par le carbone assimilé par le feuillage pour être exporté et utilisé notamment pour la formation du bois. Les informations que nous obtiendrons nous permettrons d'améliorer nos connaissances sur le transfert de carbone pour la formation du bois chez l'Hévéa. Notre hypothèse de départ est que l'allocation du carbone entre les différents compartiments de l'arbre et entre les composés

métaboliques mobiles et les composés structuraux va être affectée par les saignées, et que l'amplitude et la direction des changements qui vont s'opérer en réponse à la saignée vont dépendre de la saison. Des différences seront recherchées et reliées à la vélocité du transport phloémien et au temps de résidence du carbone dans les feuilles.

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5. Activités d'enseignement

Depuis mon recrutement à l'INRA, ma participation à l'enseignement supérieur consiste essentiellement en l'encadrement d'étudiants de tout niveau (DUT, BTS, ingénieur, master et doctorant).

Je participe aussi chaque année au fonctionnement du Master FAGE depuis sa création en tant que membre de jury au niveau Masters 1 et 2.

Enfin, Je participe ponctuellement à l'enseignement supérieur par le biais de présentations orales à l'intention de techniciens ONF (2h) de masters [57] et d'écoles chercheurs [55, 58, 60 55, 89, 96, 97, 99].

Ma participation à la vie universitaire se formalise aussi depuis quelques années par ma contribution à des comités de thèse et à un rôle d'examinateur de jury de thèse.

Enfin, en tant que membre des commissions CSE 64/68 et 66/69 à l'Université Nancy I de 2003 à 2009 j'ai été évaluatrice à plusieurs reprises de dossiers de recrutement de MCF et d'ATER.

5.1. Liste des masters encadrés

BARASCUD Y., 1999. Effets combinés de la teneur en CO_2 atmosphérique et de la fertilisation azotée sur la croissance et le métabolisme du carbone et de l'azote de jeunes chênes pédonculés (*Quercus robur* L.) cultivés en conditions contrôlées. DEA, Université Henri Poincaré Nancy I, 23 p.

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CAQUET B., 2004. Caractérisation des formes azotées impliquées dans le phénomène de poussée racinaire chez l'érable sycomore. DEA, Ecole doctorale RP2E, Université Henri Poincaré Nancy 1, 27 p.

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DESCHAZEAUX A., 1997. Effets de l'ozone sur les processus de fixation du CO_2 chez Populus tremula x alba : approches enzymatique et isotopique (δ13C). DEA, Université Henri Pointcaré Nancy I, 24 p + annexes.

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GREMLIN S., 2001. Effets combinés de la teneur en CO_2 atmosphérique et de la fertilisation azotée sur la croissance et le métabolisme du carbone et de l'azote de jeunes chênes pédonculés (*Quercus robur* L.) cultivés en conditions contrôlées. Mémoire de Maîtrise Université Henri Poincaré Nancy I. 20 p.

HOLLECOU D., 2003. Effets combinés de l'enrichissement atmosphérique en CO_2 et d'une sécheresse modérée sur le métabolisme carboné et azoté de jeunes plants de hêtre (*Fagus sylvatica* L.). Maîtrise de Sciences et Techniques de Biophysiologie appliquée aux productions végétales, UFR Sciences, 40 p.

KONATE D., 2011. Effet d'une défoliation partielle estivale et de l'ombrage sur la gestion de l'azote et du carbone au printemps chez le chêne et le hêtre âgés de 3 ans. Mémoire de Master 2 BeFage, filière CGE, Université Henri Poincaré Nancy I, 27p.

MALABOEUF K., 1999. Effets combinés du CO_2 atmosphérique et de l'azote du sol sur le métabolisme glucidique de jeunes chênes pédonculés (*Quercus robur* L.). Mémoire de Maîtrise, Université de Rennes I, 14 p + annexes.

MOUKAMBO J., 2011. Stockage et remobilisation des réserves azotées pour la croissance printanière chez le hêtre (Fagus sylvatica L.). Mémoire de Master 1 BeFage filière CGE, Université Henri Poincaré Nancy I, 15p.

MOUKAMBO J., 2012. Evolution printanière et origine des composés azotés dans les branches en croissance de chênes âgés de 50 ans. Mémoire de Master 2 BeFage, filière CGE, Université Henri Poincaré Nancy I, stage en cours.

NAVROT N., 2002. Effets interactifs de l'enrichissement atmosphérique en CO_2 et d'une sécheresse modérée sur la croissance, les échanges gazeux et le métabolisme glucidique foliaire de jeunes plants de hêtre (*Fagus sylvatica* L.) Mémoire de Maîtrise Université Henri Poincaré Nancy I, 24 p.

PICHOT T., 2000. Effets interactifs de l'enrichissement atmosphérique en CO_2 et d'une sécheresse modérée sur la croissance et les fonctionnements hydrique et trophique (carbone azote) de jeunes plants de hêtre (*Fagus sylvatica* L.). Mémoire de DEA de Biologie Forestière Université Henri Poincaré - Nancy I. 26 p. + annexes.

ROUMET C., 1992. Contribution à l'étude du métabolisme glucidique au cours de l'acquisition de l'autotrophie chez le jeune noyer. Mémoire de DEA, option morphogenèse et Production végétale, Université Blaise Pascal, Clermont III, 52 p.

5.2. Participation à l'encadrement d'étudiants en thèse

De 1990 à 1997 j'ai participé à l'encadrement des étudiants suivants pour la partie gestion du C, les marquages isotopiques et l'étude des rythmes biologiques : A. Kajji [8, 121] ; V. Pellicer [21, 134] ; C. Parmentier [20, 81, 127].

Depuis mon arrivée dans l'UMR EEF à Nancy et jusqu'à aujourd'hui j'ai participé à l'encadrement de doctorants sur différents aspects ayant trait à gestion du C et de l'N dans l'arbre et mettant en jeu des techniques biochimiques et isotopiques.

1997 à 2001 Participation à l'encadrement de Natacha Guérard (Thèse INRA Orléans, direction François Leutier et Erwin Dreyer). Mise au point et réalisation avec l'étudiante d'une expérimentation de marquage à l'aide de $^{13}CO_2$. Initiation à l'extraction, au dosage et à la purification de glucides et protéines. Discussions et participation à la rédaction scientifique. *Un article publié.*

2001 Accueil et encadrement de Sofia Cerasoli dans le cadre d'une convention Marie Curie (Thèse, Institut Supérieur d'Agronomie, Lisbonne, Portugal, direction Manuela Chaves et Joao Pereira, bourse européenne Marie Curie). Discussions scientifiques, analyses isotopiques et formation à l'extraction et la purification de composés glucidiques. *Un article publié.*

1998 à 2005 Co-encadrement Séraphine Vizoso (Thèse Université Nancy I, direction J.M. Guehl) : participation aux expérimentations (doubles marquages ^{13}C, ^{15}N ; biochimie), discussions, aide à la rédaction. *Examinateur du jury de thèse. Un article publié, un en préparation.*

2003 à 2006 Co-encadrement Luis Valenzuela (Thèse Université Nancy I, direction J.M. Guehl) avec Dominique Gérant et Nathalie Bréda. *Examinateur du jury de thèse. Deux articles publiés, un en préparation.*

2004 à 2007 Encadrement partiel de Romain Monclus (Thèse Université Orléans, direction E. Dreyer, F. Brignolas) formation aux dosages de glucides et discussions. Puis collaboration avec Cécile Barbaroux (MC, Univ. Orléans) sur le même sujet, réponses des peupliers à la sécheresse, extraction et purification de sucres solubles. Accueil de ma collègue et transfert de technique vers Orléans. *Un article en préparation*.

2009 Encadrement pendant 4 mois (Juin à septembre) de Mercedes Uscola (Thèse université Guadalajara, Espagne, direction Pr. Pedro Villar Salvador), formation sur l'isotopie et analyses isotopiques ^{13}C ^{15}N. *Une communication en 2011 et un article en préparation.*

2008 à 2010 Participation à l'encadrement de Wojnarowiecz Geneviève, Université Reims, abandon pour raisons personnelles en fin de thèse (2006-2009). Direction : Pr. Christophe Clément. *Un article publié, deux posters*.

2007 à 2011 Co-encadrement Rana El Zein (Thèse Université Nancy I, direction N. Breda). Participation aux campagnes d'échantillonnage, à la conception des expériences, aux discussions et à la rédaction. *Deux articles publiés, un soumis.*

5.3. Participation à l'encadrement de post-doctorants

1994 Abraham Escobar-Gutierrez (*Deux articles, deux posters*)

2009 Pierre Emmanuel Courty (*Une communication orale, un article soumis*)

5.4. Membre de Jury de thèse en tant qu'examinateur

2004 Séraphine Vizoso

2006 Luis Valenzuela Nunes

5.5. Membre de comités de thèse

BARBAROUX C., 2002. Analyse et modélisation des flux de carbone de peuplements forestiers pour la compréhension de la croissance de deux espèces feuillues *Quercus petraea* et *Fagus sylvatica*. Thèse de Doct. en Sci., Univ. Paris-Sud, Orsay, 178 p. + annexes.

BOUTEBTOUB Wahiba, 2010. Etude comparative de la gestion carbonée du Mandevilla sanderi chez une plante conduite en libre cours et une plante qui subit des traumatismes successifs de taille. Thèse de Doctorat, Spécialité Sciences Agronomiques, Ecole doctorale d'Angers.

DELAIRE Mickaël, 2005. Variations de la capacité d'absorption minérale par les racines du jeune Acer pseudoplatanus, L. (Acéracées) consécutives à l'histoire nutritionnelle récente et ancienne de la plante. Application à la culture hors sol des végétaux ligneux. Thèse de Doctorat, Spécialité Sciences Agronomiques, Ecole doctorale d'Angers, 177p.

EGLIN Thomas, 2008. Le déterminisme climatique et physiologique des variations saisonnières de la signature isotopique de la matière organique au sein d'arbre décidus. Thèse de Doct. en Sci., Univ. Paris-Sud, Orsay, 223 p.

GILSON Angélique, en cours. Etude du fonctionnement couplé carbone-azote dans les écosystèmes forestiers. Thèse de Doct. en Sci., Univ. Paris-Sud, Orsay, ESE.

PELLICER V., 1997. Etude de l'influence de facteurs environnementaux et physiologiques sur l'aptitude à l'enracinement de boutures de mélèze hybride (Larix x eurolepis Henry) Thèse de Doctorat, Université d'Angers, Nogent sur Vernisson, France, Centre National du Machinisme Agricole, du Génie Rural, des Eaux et des Forêts, 206 p.

PLUCHON Morgane, 2010-2013. Dynamiques saisonnières des réserves carbonées et azotées chez le peuplier et l'eucalyptus en plantation pure et en association avec des espèces

fixatrices d'azote. Encadrement D. Epron et D. Gérant, Ecole doctorale RP2 Université Nancy I.

RICHET N., 2010. Effet des contraintes environnementales (ozone et fort CO2) sur le bois des jeunes arbres : étude de la formation du bois et conséquences sur ses propriétés en tant que matériau. Thèse de Doctorat de l'Université Henri Poincaré, Ecole doctorale RP2E, 187 p

WOJNAROWIEZ Geneviève, Université Reims, abandon pour raisons personnelles en fin de thèse (2006-2009). Direction : Pr. Christophe Clément.

6. Activités d'administration, de valorisation et de transfert

6.1. Activités d'administration

6.1.1. UMR 1137

Mes activités sont essentiellement scientifiques et je n'ai pas eu de tâches administratives lourdes au sein même de mon UMR. J'ai cependant participé au développement des outils analytiques pour pouvoir exercer mon activité scientifique, en faisant notamment une demande de matériel moyen en 1996 et 1997 peu de temps après mon arrivée à Nancy. Puis j'ai monté un laboratoire de biochimie opérationnel dans une aile nouvellement construite, avec tout l'équipement nécessaire à l'extraction et au dosage de différents composés (chlorophylles, glucides, acides aminés, protéines), à savoir un évaporateur sous vide, une ultra-centrifugeuse réfrigérée, un lyophilisateur semi-industriel, un spectrophotomètre Beckman, des congélateurs et ultra-congélateurs, un lave-vaisselle, etc. ... L'ensemble de ce laboratoire, toujours opérationnel et de plus en plus sollicité par mes collègues, appartient depuis quelques années maintenant au PTEF dirigé par Nicolas Angeli.

J'ai également fait partie du conseil scientifique de service de l'UMR 1137 dans ses premières années d'existence.

6.1.2. Départements EA et EFPA à l'INRA

En tant que membre élu du conseil scientifique du département Environnement et Agronomie de 2002 à 2006, j'ai participé aux activités de mon Institut à diverses reprises. Dans le cadre de cette fonction je suis intervenue en tant que représentante du Conseil Scientifique et expert nommé pour évaluer plusieurs unités mixtes de recherche, ceci à la demande de mon chef de département Laurent Bruckler (aujourd'hui président du centre INRA de Montpellier). A chaque évaluation d'Unités mixtes INRA-Université (Ephyse en 2003, Oenologie-Ampélologie à Bordeaux 2006, PSH à Avignon en 2007) il nous a été demandé de faire un compte-rendu oral puis écrit avec nos conseils et recommandations pour les années à venir. J'ai aussi été désignée par mon chef de département chaque année comme expert de projets innovants sur la période 2002-2006. J'ai ensuite changé de département et ai été sollicitée à nouveau en 2010 par mon chef de département EFPA (Jean-Marc Guehl) pour ce type d'expertise.

En 2011 j'ai été élue en tant que représentant du personnel aux Commissions Administratives Paritaires Nationales des chargés de recherche à l'INRA (CAPN, je suis membre suppléant de Laurent Ferrier, INRA Toulouse). La CAPN peut être consultée par la

direction des ressources humaines de l'INRA sur toute question individuelle, relative à la situation professionnelle des personnels titulaires et, obligatoirement, sur les décisions individuelles disciplinaires et de licenciement.

6.1.3. Université Nancy I

Au sein de l'Université de Nancy, j'ai exercé plusieurs années de suite et ce jusqu'en 2009 en tant que membre de deux commissions de spécialistes (64/68 : président Pr. J. Brun Bellut ; 66/69 : président Pr Chalot) et ai participé au recrutement de plusieurs maîtres de conférence et personnels Ater pendant cette période.

Dans le cadre de la Société française de Physiologie végétale affiliée à la FESPP, j'ai été membre élu du Conseil de la Société française de Physiologie végétale affiliée à la FESPP plusieurs années et participé à son fonctionnement jusqu'en 2008.

6.2. Activités de valorisation et de transfert et rayonnement scientifique

Dans le cadre de l'AIP Ecofon soumis à l'INRA par Raymond Bonhomme et dont le financement a été accepté et financé de 1994 à 1997, j'ai participé au transfert de connaissances en organisant deux colloques nationaux en 1993 et 1998 à Paris, concernant (1) l'analyse des régulations des structures aériennes et racinaires des plantes et des fonctions physiologiques sous l'effet de contraintes environnementales physico-chimiques et biologiques, et (2) l'élaboration de méthodologies spécifiques pour caractériser des structures avec un suivi quantitatif à long-terme des compartiments carbonés et azotés, et une intégration de processus dans des modèles dynamiques de fonctionnement des cultures. Suite à ces deux colloques j'ai ensuite édité avec mon collègue R. Bonhomme les travaux les plus significatifs dans deux ouvrages INRA de la série colloques.

Les réserves des tissus végétaux spécialisés tels que graines, bulbes, tubercules, ont fait l'objet de travaux pionniers, celles intervenant dans des tissus plus généralistes (feuilles, racines, troncs…), n'ont fait l'objet de travaux que plus tardivement bien que leur rôle sur le fonctionnement trophique du végétal s'avère essentiel. Aussi, en 2009, j'ai organisé avec mon collègue le Pr. Alain Ourry (INRA, EVA Caen) et son équipe un colloque franco-anglophone dont la vocation était de dresser un bilan sur l'état des connaissances, des méthodologies et des perspectives relatives à ces réserves des tissus végétatifs des plantes de grande culture mais aussi de plantes ligneuses et plus particulièrement des arbres. Ce colloque a été financé majoritairement par les départements EA et EFPA de l'INRA, et l'Université de Caen. Deux personnalités étrangères phare du domaine ont été invitées pour présenter les réserves des

espèces ligneuses (P. Millard, MLURI, Aberdeen, GB) et herbacées (J.J. Volenec, Purdue University, USA).

En 2010, j'ai participé à l'organisation d'un workshop qui réunissait des partenaires de différents organismes de recherche français (INRA, CNRS, CIRAD) et étrangers (Brésil) avec plusieurs de mes collègues. Ce groupe de travail s'est penché sur l'aide que peuvent apporter les isotopes stables pour étudier la fixation de l'N et le recyclage de l'N dans les écosystèmes" et l'état de l'art concernant la méthodologie et les modèles utilisés pour estimer la fixation de l'azote et le cycle de l'azote a été inventorié, tant au niveau des plantes que des écosystèmes. Les isotopes stables sont des traceurs non radioactifs précieux en milieu naturel pour étudier l'acquisition des ressources par une plante ou par les micro-organismes, ainsi que l'allocation des ressources dans une plante, entre ses différents organes et fonctions métaboliques.

Fin 2010 et en 2011 j'ai présenté une partie des travaux de thèse de Rana El Zein au en Suède [41] et au Japon [42]. En tant qu'expert en marquage isotopique et gestion du C et de l'N dans les arbres, j'ai été sollicitée en 2010 et 2011 pour participer à un projet finlandais concernant la gestion du C et de l'N de jeunes arbres en forêt boréale soumis à une hypoxie racinaire hivernale (encapsulage des racines dans la glace) (coordination du projet, Minna Turunen, Arctic Centre, University of Lapland, Finland).

En septembre 2012 je vais participer à une formation aux isotopes stables dans les recherches sur les écosystèmes (Ecost Sibae, 23-29 Sep 2012, Nancy, France).

Je suis aussi sollicitée de temps à autres en tant qu'isotopiste par des collègues d'autres disciplines scientifiques. J'ai ainsi récemment collaboré avec Florian Mermillod-Blondin et Laurent Simon (UMR-CNRS5023, LEHF Université Lyon I) sur un sujet ayant trait au métabolisme nutritionnel de crustacés aquatiques, en réalisant avec eux un double marquage ^{13}C ^{15}N d'aulnes glutineux début 2011, les feuilles d'aulne étant la principale source d'alimentation de ces crustacés. L'objectif était de suivre les traceurs de l'aulne au crustacé et de comprendre les étapes métaboliques et le devenir du C et de l'N dans l'organisme aquatique.

Mes travaux en gestion du C et de l'N et en isotopie ont aussi été la source d'une collaboration téléphonique et par email avec Céline Leroy en post doctorat (EcoFoG, Ecologie des Forêts de Guyane, UMR-CNRS 8172, Campus Agronomique, 97379 Kourou Cedex, France) afin de l'aider à réaliser une expérimentation de marquage lui permettant de déterminer via des marquages en azote enrichi d'*Aechmea mertensii* abandonnés si 1) oui ou non l'azote est assimilé par les racines d'un part et les feuilles d'autre part et 2) pouvoir déterminer la part assimilée par les racines et les feuilles. L'*Aechmea mertensii* est une

bromeliacée épiphyte qui forme des réservoirs. La particularité de cette broméliacée est qu'elle est strictement présente dans les jardins de fourmis.

7. Liste des publications

7.1. Articles scientifiques (revues avec comité de lecture)

1. CERASOLI S., **MAILLARD P.**, SCARTAZZA A., BRUGNOLI E., CHAVES M.M., PEREIRA J.S., 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork oak (*Quercus suber* L.) saplings. *Annals of Forest Science*, 61: 721–729.

2. DANNOURA M., **MAILLARD P.**, FRESNEAU C., PLAIN C., BERVEILLER D., GERANT D., CHIPEAUX C., BOSC A., NGAO J., DAMESIN C., LOUSTAU D., EPRON D., 2011. *In situ* assessment of the velocity of carbon transfer by tracing ^{13}C in trunk CO_2 efflux after pulse labelling: variations among tree species and season. *New Phytologist*, 190: 181-192.

3. DAUDET F.A., LACOINTE A., **MAILLARD P.**, ESCOBAR-GUTIERREZ A.J., DELEENS E., 2001. A mechanistic and dynamic model of carbon allocation in walnut (*Juglans regia* L.) seedlings. *Acta Horticulturae*, 544: 209-217.

4. ESCOBAR-GUTIERREZ A.J., DAUDET F.A., GAUDILLERE J.P., **MAILLARD P.**, FROSSARD J.S., 1998. Modelling of allocation and balance of carbon in walnut (*Juglans regia* L.) seedlings during heterotrophy-autotrophy transition. *Journal of Theoretical Biology*, 194: 29-47.

5. EL ZEIN R., BREDA N., GERANT D., ZELLER B., **MAILLARD P.**, 2011. Nitrogen sources for current-year shoot growth in 50-year-old sessile oak trees: An in situ ^{15}N labeling approach. *Tree Physiology*, vol. 31, no. 12, pp. 1390–1400.

6. EL ZEIN R., **MAILLARD P.**, BREDA N., MARCHAND J., MONTPIED P., GERANT D., 2011. Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. *Tree Physiology*, 31: 843–854.

7. GUERARD N., **MAILLARD P.**, BRECHET C., LIEUTIER F., DREYER E., 2007. Do trees use reserve or newly assimilated carbon for their defense reactions? A ^{13}C labeling approach with young Scots pines inoculated with a bark-beetle-associated fungus (*Ophiostoma brunneo ciliatum*). *Annals of Forest Sciences*, 64: 601-608.

8. KAJJI A., LACOINTE A., DAUDET F.A., **MAILLARD P.**, ARCHER P., 1990. Carbon economy of a young walnut tree. *Acta Horticulturae*, 284: 223-232.

9. **MAILLARD P.**, DELEENS E., CASTELL F., DAUDET F.A., 1999. Source-sink relationships for carbon and nitrogen during early growth of *Juglans regia* L. seedlings: analysis at two elevated CO_2 concentrations. *Annals of. Forest Sciences*, 56: 59-69.

10. **MAILLARD P.**, DELEENS E., DAUDET F.A., LACOINTE A., FROSSARD J.S., 1994. Carbon economy in Walnut seedlings during the acquisition of autotrophy studied by long-term labelling with $^{13}CO_2$. *Physiologia Plantarum*, 91: 359–368.

11. **MAILLARD P.**, DELEENS E., DAUDET F.A., LACOINTE A., FROSSARD J.S., 1994. Carbon and nitrogen partitioning in walnut seedlings during the acquisition of

autotrophy through simultaneous $^{13}CO_2$ and $^{15}NO_3$ long-term labelling. *Journal of Experimental Botany*, 45: 203–210.

12. **MAILLARD P.**, GUEHL J.M., MULLER J.F., GROSS P., 2001. Interactive effects of elevated CO_2 concentration and nitrogen supply on partitioning of newly fixed ^{13}C and ^{15}N between shoot and roots of pedunculate oak seedlings (*Quercus robur*). *Tree Physiology*, 21: 163-172.

13. **MAILLARD P.**, GARRIOU D., DELEENS E., GROSS P., GUEHL J.M., 2004. The effects of lifting on mobilisation and new assimilation of C and N during regrowth of transplanted Corsican pine seedlings. A dual ^{13}C and ^{15}N labelling approach. *Annals of Forest Sciences*, 61: 795-805.

14. **MAILLARD P.**, JACQUES M., MIGINIAC E., JACQUES R., 1987a. Croissance de jeunes *Terminalia superba* en conditions contrôlées. *Annales des Sciences Forestières*, 44 : 67 – 84.

15. **MAILLARD P.**, JACQUES M., MIGINIAC E., 1987b. Correlative growth in young *Terminalia superba* in a controlled environment: effect of the leaves on internode elongation. *Annals of Botany*, 60: 447-454.

16. **MAILLARD P.**, JACQUES M., MIGINIAC E., MILLET B., 1989a. Mathematical analysis and comparison of growth fluctuations of the aerial system of young *Terminalia superba*, Englers et Diels (*Combretaceae*). International Symposium Forest Tree Physiology ; 1988/09/25-30 ; Nancy (FRA). *Annales des Sciences Forestières*, 46 (suppl.): 202s-205s.

17. **MAILLARD P.**, JACQUES M., MIGINIAC E., MILLET B., 1989b. Mathematical growth analysis of young *Terminalia superba* plants in a controlled environment: comparison of growth rhythms of the principal axis and of axillary branches. *Annals of Botany*, 64: 95-105.

18. NUNEZ L.M.V., GERANT D., **MAILLARD P.**, BREDA N., 2010. Seasonal dynamics of total soluble proteins in adult trees of *Quercus petraea* (Matts.) Liebl. *Revista Mexicana de Ciencas Forestales*, ISSN: 2007-1132, Vol 1, No 1., 75-83.

19. NUNEZ L.M.V., GERANT D., **MAILLARD P.**, BREDA N., CERVANTES G.G., COHEN I.S., 2011. Evidence for a 26kDa vegetative storage protein in the stem sapwood of mature pedunculate oak. *Interciencia*, 36 (2), 142-147.

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21. PELLICER V., GUEHL JM, DAUDET FA, CAZET M., RIVIERE LM, **MAILLARD P.**, 2000. Carbon and nitrogen mobilisation in *Larix* × *eurolepis* leafy stem cuttings assessed by dual C-13, N-15 labeling – relationships with rooting. *Tree Physiology*, 20: 807–814.

22. PLAIN C., GERANT D., **MAILLARD P.**, DANNOURA M., DONG Y., ZELLER B., PRIAULT P., PARENT F., EPRON D., 2009. Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ $^{13}CO_2$ pulse labelling of 20-year-old beech trees. *Tree Physiology*, 29: 1433-1445.

23. RICHET N., AFIF D., TOZO K., POLLET B., **MAILLARD P.**, HUBER F., PRIAULT P., BANVOY J., GROSS P., DIZENGREMEL P., LAPIERRE C., PERRE P., CABANE M., 2012. Elevated CO_2 and/or ozone modify lignification in the wood of poplars (Populus tremula x alba). *Journal of Experimental Botany*, sous presse.

24. VAILLANT-GAVEAU N., **MAILLARD P.**, WOJNAROVIECZ G., GROSS P., FONTAINE F., 2011. Inflorescence of grapevine (*Vitis vinifera* L.): a high ability to distribute its own assimilates. *Journal of Experimental Botany*, 62: 4183–4190.

25. VIZOSO S., GERANT D., GUEHL J.M., JOFFRE R., CHALOT M., **MAILLARD P.**, 2008. Do elevation of CO_2 concentration and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings? *Tree Physiology*, 28: 1729-1739.

7.2. Articles soumis

26. BREDA N., **MAILLARD P.**, MONTPIED P., BRECHET C., GARBAYE J., COURTY P.E., 2012. Isotopic evidence in adult oak trees of a mycoheterotrophic lifestyle during spring. *Functional Ecology*, soumis.

27. GERANT D., EL ZEIN R., **MAILLARD P.**, MONTPIED P., GEREMIA F., BREDA N., 2012. Accumulation and storage of non-structural carbon and nitrogen pools in 50-year-old sessile oak and beech trees. Invited issue of Tree Physiology on Carbon Allocation of Trees and Forests. *Tree Physiology*, soumis.

28. EPRON D., BAHN M., DERRIEN D., LATTANZI A. F., PUMPANEN J., GESSLER A., HÖGBERG P., **MAILLARD P.**, DANNOURA M., GERANT D., BUCHMANN N., 2012. Labelling trees to study carbon allocation patterns: a review of methods, current knowledge and future prospects. Invited issue of Tree Physiology on Carbon Allocation of Trees and Forests. *Tree Physiology*, soumis.

7.3. Articles en préparation

29. AFIF D., **MAILLARD P.**, RUMEAU D., DESCHAZEAUX A., DIZENGREMEL P., GUEHL J.M., en préparation.The effects of ozone on leaf gas exchange, CO_2 fixation and whole plant ^{13}C allocation in poplar.

30. **MAILLARD P.**, GERANT D., NUNEZ L.M.V., MARCHAND J.., BRECHET C., BREDA N., en préparation. Shading and defoliation in summer alter spring N remobilisation and N uptake in *Quercus petraea* and *Fagus sylvatica* L. saplings.

31. **MAILLARD P.**, TRIBOULOT-BOGEAT M.B., GENERAL E., CHURIN J.L., GARBAYE J., en préparation. Outplanting performance of bare-root sessile oak seedlings in relation with mycorrhizal status and storage conditions.

7.4. Communications dans des congrès et colloques

32. AFIF D., **MAILLARD P.**, DIZENGREMEL P., GUEHL J.M., 2001. Assimilation et allocation du carbone chez le peuplier en réponse à une contrainte environnementale l'ozone. Réunion du Réseau d'Ecophysiologie de l'Arbre. Autrans 16-19 janvier 2001. ***Poster et résumé***.

33. AMEGLIO T., ARCHER P., CRUIZIAT P., DAUDET F.A., FROSSARD J.S., KAJJI A., LACOINTE A., **MAILLARD P.**, 1995. Carbon economy and water relations in a 3 year-old walnut: physiological interactions. III International Walnut Congress, Alcobaça (P), June 13-16 1995. ***Poster***.

34. BOGEAT-TRIBOULOT M.B., PICHOT T., **MAILLARD P.**, 2001. Effet de l'élévation de [CO_2]atm et d'une sécheresse modérée sur les propriétés hydrauliques des racines de jeunes hêtres. Réunion du Réseau d'Ecophysiologie de l'Arbre. Autrans 16-19 janvier 2001. ***Poster et résumé***.

35. CASTELL F., DAUDET F.A., **MAILLARD P.**, 1994. Early effects of increased CO_2 on growth and metabolism of young walnut. Colloque ECOCRAFT, Dourdan, 19-21 Octobre 1994. ***Poster***.

36. CHENEVARD D., FROSSARD J.S., JAY-ALLEMAND C., LACOINTE A., **MAILLARD P.**, GENDRAUD M., 1994. Carbon partitioning in walnut seedlings and microcuttings during the acquisition of autotrophy. CEE AIR3 Walnut n° CT92-0142, General Workshop , Orléans (F), March 23-26 1994. ***Communication orale en anglais***.

37. DAUDET F.A., **MAILLARD P.**, 1998. Etude et modélisation des règles d'allocation des assimilats dans les plantes en relation avec l'environnement. Caractéristiques physiologiques et régulation du transport des assimilats C et N. Réunion de fin d'AIP « ECOFON » organisée par R. Bonhomme, Janvier 1998, Paris INAPG. ***Communication orale***.

38. ESCOBAR-GIUTERREZ A., **MAILLARD P.**, DAUDET F.A., 1996. Modelling allocation and balance of carbon in walnut (*Juglans regia* L.) seedlings during the heterotrophy-autotrophy transition period. International conference on the transport of photoassimilates, University of Kent, Canterbury, U.K., 13-17 Août 1995. Journal of Experimental Botany, Special Issue, 47, 1320. ***Poster***.

39. ESCOBAR GUTIERREZ A., **MAILLARD P.**, LAHANQUE X., DAUDET F.A., FROSSARD J.S., 1995. Utilisation de SPICE pour la simulation des flux et du bilan de C dans les jeunes plants de noyer (*Juglans regia* L.). III colloque jeunes chercheurs SFPV, Clermont-Ferrand (F), 6-8 septembre 1995. ***Poster***.

40. ESCOBAR GUTIERREZ A., DAUDET F.A., **MAILLARD P.**, GAUDILLERE J.P., FROSSARD J.S., 1998. Deterministic and dynamic modelling of carbon allocation within walnut seedlings (*Juglans regia* L.). 2nd international workshop on Functional-Structural Tree Models, Clermont-Ferrand, 12-15 Octobre 1998, p. 45. ***Poster***.

41. EL ZEIN R., BREDA N., GERANT D., ZELLER B., **MAILLARD P.**, 2011. N sources for current-year shoot growth in 50-year-old sessile oak trees Nitrogen workshop by Tobias Rütting *et al.* "Advances in labelling and analysis approaches in ^{15}N enrichment studies" was organized together with eCOST SIBAE and took place 09-11 May 2011 at University of Gothenburg, Sweden. ***Communication orale en anglais***.

42. EL ZEIN R., GERANT D., BREDA N., ZELLER B., **MAILLARD P.**, 2010. N source for spring branch growth in 50 year-old-oaks: a ^{15}N labelling approach. Symposium on the use of new techniques to understand gas exchange and carbon dynamics in the forest ecosystem", 01 November 2010, Faculty of Agriculture, Kyoto University, Japan. ***Communication orale en anglais***.

43. EPRON D., DANNOURA M., NGAO J., PLAIN C., BERVELLER D., CHIPEAUX C., GERANT D., BOSC A., **MAILLARD P.**, LOUSTAU D., DAMESIN C., 2010. Seasonal variations of the amount of carbon allocated to respiration after *in situ* $^{13}CO_2$ pulse labelling of trees. American Geophysical Union, Fall Meeting 2010, San Francisco (Invited, Tree Physiology Lecture), abstract #B12B-01, ***Communication orale en anglais***.

44. EPRON D., DANNOURA M., NGAO J., PLAIN C., BERVELLER D., CHIPEAUX C., GERANT D., BOSC A., **MAILLARD P.**, LOUSTAU D., DAMESIN C. 2010. Seasonal variations of the amount of carbon allocated to respiration after in situ $^{13}CO_2$ pulse labelling of trees. Symposium on the usage of new techniques to understand gas exchange and carbon dynamics in the forest ecosystem, Kyoto, Japan, 1 November 2010. ***Communication orale en anglais***.

45. FALCIMAGNE R., MARTIGNAC M., **MAILLARD P.**, 1990. Gestion automatique d'une enceinte de mesure d'échanges gazeux à deux compartiments. Dans : Les journées de la mesure électronique, informatique, automatique. Port Leucate, 3-4 octobre 1990, II-36-II-39. ***Communication orale***.

46. FROSSARD J.S., **MAILLARD P.**, CHENEVARD D., DELEENS E., DAUDET F.A., LACOINTE A., 1995. Growth and partitioning of carbon and nitrogen in walnut during germination and seedling growth. III International Walnut Congress, Alcobaça (P), June 13-16 1995. ***Communication orale en anglais***.

47. GARBAYE J., BREDA N., MONTPIED P., BRECHET C., **MAILLARD P.**, COURTY P.E., 2011. Can trees be mycoherotrophic? Isotopic evidence of soil carbon acquisition by ectomycorrhizal oak roots during spring reactivation. 12th EEF Congress 2011. The European Ecological Federation (EEF, www.europeanecology.org) Ávila, Spain, from 25 till 29 September in 2011. ***Communication orale en anglais***.

48. GARRIOU D., **MAILLARD P.**, DELEENS E., GUEHL J.M., 1996. Acquisition and allocation of ^{15}N in forest tree seedlings after transplanting. "Informal European Stable Isotope Meeting III", CRPG Nancy, France, 30 sept - 1 oct 1996. ***Poster***.

49. GERANT D., **MAILLARD P.**, BREDA N., VIZOSO S., VALENZUELA NUNES L., GENET H., El ZEIN R., EPRON D., PLAIN C., 2009. Intra-tree distribution and seasonal dynamics of carbon and nitrogen storage compounds in young and mature temperate trees. Tools for characterization and quantification. " Les réserves végétales et leur importance agronomique et sylvicole "Université de Caen Basse-Normandie 9 et 10 Juin 2009. ***Communication orale***.

50. GERANT D., VALENZUELA NUNES L., BREDA N., BARBAROUX C., **MAILLARD P.**, VIZOSO S., DIZENGREMEL P., GUEHL J.M., 2005. La thématique « réserves carbonées et azotées » de l'UMR 1137 Ecophysiologie et Ecologie Forestières INRA Nancy - UHP Nancy I. Conférence dans le cadre de la formation des

Ingénieurs ONF ou d'administrations forestières organisée par l'UMR. « Présentation des concepts de l'écologie fonctionnelle et de l'écophysiologie forestières et des programmes de recherche ». INRA Champenoux, Mars 2005. ***Communication orale***.

51. GERANT D., VALENZUELA NUNES L., BREDA N., BARBAROUX C., **MAILLARD P.**, VIZOSO S., DIZENGREMEL P., GUEHL J.M., 2004. Dynamique saisonnière du carbone et de l'azote chez le chêne et le hêtre : mise en réserve et remobilisation. Conférences sur invitation dans le cadre d'une mission de coopération France-Canada : 14 décembre 2004 au Centre de Recherche en Biologie Forestière (CRBF), Pavillon Marchand, Université Laval, Québec et 17 décembre 2004 au Groupe de Recherche en Ecologie Forestière (GREF), UQAM, Montréal. Décembre 2004. ***Communication orale***.

52. GERANT D., VALENZUELA NUNES L., BREDA N., BARBAROUX C., **MAILLARD P.**, VIZOSO S., DIZENGREMEL P., GUEHL J.M., 2005. La thématique « réserves carbonées et azotées » de l'UMR 1137 Ecophysiologie et Ecologie Forestières INRA Nancy - UHP Nancy I. Conférence dans le cadre de la formation des Ingénieurs ONF ou d'administrations forestières organisée par l'UMR. « Présentation des concepts de l'écologie fonctionnelle et de l'écophysiologie forestières et des programmes de recherche ». INRA Champenoux, Mars 2005. ***Communication orale***.

53. HOLLECOU D., **MAILLARD P.,** GERANT D., VIZOSO S., 2003. Effets combinés de l'enrichissement atmosphérique en CO_2 et d'une sécheresse modérée sur les métabolismes carboné et azoté de jeunes plants de hêtre (*Fagus sylvatica* L.). V èmes Journées d'Ecologie Fonctionnelle, Nancy, France, Mars 2003. ***Poster***.

54. KAJJI A., LACOINTE A., DAUDET F.A., **MAILLARD P.**, ARCHER P., 1989. Carbon economy of a young walnut tree. Dans : International Symposium on walnut production. Budapest, Hongrie, 25-29 Septembre 1989.

55. **MAILLARD P.**, 2001. NETCARB project summer school 26-29 Septembre 2001 Orvieto Italie. "A simple method for extraction and purification of total soluble proteins". ***Communication orale en anglais***.

56. **MAILLARD P.**, 2001. Conséquences des changements climatiques sur la croissance et le fonctionnement trophique d'espèces forestières d'intérêt ». Réunion CAFQA animée par B. Seguin (dép. Environnement et Agronomie INRA.) 25 Avril 2001 INRA Champenoux. ***Communication orale***.

57. **MAILLARD P.**, 2003, 2004 et 2005. « Les impacts de l'augmentation du CO_2 atmosphérique sur les arbres forestier avec visite des serres à CO_2 ». Formation continue CNFF « La forêt et les modifications environnementales d'origine humaine : CO2, pollution, climat », 24-25 juin 2003, INRA Champenoux. ***Communication orale***.

58. **MAILLARD P.**, 2003. Ecole Chercheurs "Intégration de processus à l'échelle de la plante entière : Interactions entre les gestions de l'azote, de carbone et de l'eau". 12 au 16 mai 2003 à Saint-Flour, Cantal, France. ***Communication orale***.

59. **MAILLARD P.**, 2003. « Le fonctionnement carboné et azoté chez les arbres ». Exposé Maîtrise Université Lilles, Avril 2003, INRA Champenoux. ***Communication orale***.

60. **MAILLARD P.**, 2009. Using ^{13}C and ^{15}N labelling to investigate and N dynamics in change in storage compounds. Training course on the use of stable isotopes in forest ecology Champenoux, March 2009 (Evoltree, nfz forestnet, …). ***Communication orale en anglais. Publication sous forme de DVD***.

61. **MAILLARD P.**, GUEHL J.M., 1998. Effets combinés de la fertilisation azotée et de l'augmentation de la concentration atmosphérique en CO_2 sur la croissance du chêne pédonculé. Conférence ENSTIB 21-23 Septembre 1998, Epinal, 2èmes journées scientifiques Forêt-Bois. ***Communication orale***.

62. **MAILLARD P.**, ROCHER J.P., 1990. Effect of light on the regulation of carbon partitioing in mature leaves of *Zea mays* L.. Dans: International Conference on Phloem Transport and Assimilate Compartmentation, Cognac, France, 19-24 août 1990, p. 92. ***Poster***.

63. **MAILLARD P.**, DELEENS E., CASABIANCA H., 1993. Etude à l'aide d'un double marquage à long terme $^{13}C/^{15}N$ de la répartition du carbone et de l'azote chez de jeunes noyers lors de l'acquisition de l'autotrophie. Dans: Les Isotopes Stables en Recherche Agronomique Végétale. Séminaire I.N.R.A., 16 et 17 Décembre 1993, INA-PG, Paris, France. ***Communication orale***.

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65. **MAILLARD P.**, PICON-COCHARD C., GUEHL J.M., 1998. Effets combinés d'un doublement de la concentration de CO_2 de l'air et d'une sécheresse sur l'écophysiologie des chênes sessile et pédonculé. Programme de la Réunion du Groupe Chêne, INRA Bordeaux, 21-22-23 avril 1998. ***Communication orale***.

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67. **MAILLARD P.**, VIZOSO S., GUEHL J.M., 2001. Effets combinés des teneurs en CO_2 atmosphérique et en azote du sol sur l'assimilation de carbone et d'azote par des plants de chêne pédonculé. Implications pour la croissance et la mise en réserve ». Premières Rencontres d'Ecophysiologie de l'Arbre Ecole Thématique16 - 19 Janvier 2001 Autrans (Isère). ***Communication orale***.

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Colloque National de la Société Française de Physiologie Végétale. La Colle -sur-Loup (France), 16-18 Octobre 1991. ***Poster et résumé***.

70. **MAILLARD P.**, VALENZUELA NUNES L., GERANT D., BRECHET C., EL ZEIN R., BREDA N., 2008. Comparison of spring 15N uptake in oak and beech saplings submitted to shading and summer defoliation. Session 3-Poster, JESIUM 2008, Presqu'île de Giens, France, August 31-September 5, Final programme& abstract book, 3-P106, page 140. ***Poster et résumé***.

71. **MAILLARD P.**, PICHOT T., TRIBOULOT-BOGEAT M.B., WILLM F., GUEHL J.M., 2000. Will elevated [CO_2] combined with a moderate drought alter water/carbon/nitrogen relations in young beech (*Fagus sylvatica* L.) trees? 4ème colloque général de la Société Française de Physiologie Végétale. Besançon 5-7 décembre 2000. ***Poster et résumé***.

72. **MAILLARD P.**, PICHOT T., TRIBOULOT-BOGEAT M.B., GERANT D., BREDA N., 2011. Interactive effects of atmospheric CO_2 enrichment and of a moderate drought on growth, water-use efficiency and ^{15}N uptake in beech (*Fagus sylvatica* L.) seedlings. "Managed forest in future landscape, implication for water and carbon cycles" Congrès COST, Santiago de compostela, May 8-11 2011. ***Poster et communication orale en anglais***.

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Do elevation of CO_2 concentration and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings?

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Summary Soil nitrogen can alter storage and remobilization of carbon and nitrogen in forest trees and affect growth responses to elevated carbon dioxide concentration ([CO_2]). We investigated these effects in oak saplings (*Quercus robur* L.) exposed for two years to ambient or twice ambient [CO_2] in combination with low- (LN, 0.6 mmol N l^{-1}) or high-nitrogen (HN, 6.1 mmol N l^{-1}) fertilization. Autumn N retranslocation efficiency from senescing leaves was less in HN saplings than in LN saplings, but about 15% of sapling N was lost to the litter. During the dormant season, nonstructural carbohydrates made up 20 to 30% of the dry mass of perennial organs. Starch was stored mainly in large roots where it represented 35–46% of dry mass. Accumulation of starch increased in large roots in response to LN but was unaffected by elevated [CO_2]. The HN treatment resulted in high concentrations of N-soluble compounds, and this effect was reduced by elevated [CO_2], which decreased soluble protein N (–17%) and amino acid N (–37%) concentrations in the HN saplings. Carbon and N reserves were labeled with ^{13}C and ^{15}N, respectively, at the end of the first year. In the second year, about 20% of labeled C and 50% of labeled N was remobilized for spring growth in all treatments. At the end of leaf expansion, 50–60% of C in HN saplings originated from assimilation versus only 10–20% in LN saplings. In HN saplings only, N uptake occurred, and some newly assimilated N was allocated to new shoots. Through effects on the C and N content of perennial organs, elevated [CO_2] and HN increased remobilization capacity, thereby supporting multiple shoot flushes, which increased leaf area and subsequent C acquisition in a positive feedback loop.

Keywords: ^{13}C, ^{15}N, carbon remobilization, carbon storage, elevated CO_2, nitrogen remobilization, nitrogen storage, Quercus robur.

Introduction

Elevation of atmospheric carbon dioxide concentration ([CO_2]) can enhance plant growth, thereby increasing the demand for mineral nutrients (Ceulemans and Mousseau 1994, Stitt and Krapp 1999). Responses to elevated [CO_2] vary depending on nutrient availability and species. Given the importance of nitrogen limitation on the growth responses of temperate forests and the increase in N deposition (Bauer et al. 2001), the effect of soil N availability on tree responses to increasing atmospheric [CO_2] is of particular interest. Positive effects of elevated [CO_2] on carbon assimilation and plant growth are often limited on N-poor soils, as shown in *Quercus virginiana* Mill., *Quercus ilex* L. and *Quercus robur* L. (Tognetti and Johnson 1999*a*, 1999*b*, Maillard et al. 2001). In contrast, increasing N supplies can significantly enhance growth responses of trees to elevated [CO_2] and modify C and N dynamics (Bauer et al. 2001, Maillard et al. 2001, Dyckmans and Flessa 2002).

Unlike annual plants, which respond to a CO_2-enriched atmosphere without carry-over effects of an earlier lower [CO_2], except as mediated by an effect on seeds, perennial organs of trees may show cumulative effects of elevated [CO_2] (Körner 2006). Internal reserves of stems and roots play an essential role during winter and early spring by supplying C and N for both maintenance metabolism and new growth, and their seasonal dynamics are central to the responses of trees to elevated [CO_2].

Nitrogen availability during growth has marked effects on development and N storage capacity of temperate trees (Stephen et al. 2001). Although the effects of elevated [CO_2] on soil-N uptake during the growing season have been documented, there have been few studies on N remobilization in trees growing in an elevated [CO_2] atmosphere. Increased growth and storage sink capacity in response to elevated [CO_2] may increase the extent of N storage and remobilization in several tree species (Temperton et al. 2003, Dyckmans and Flessa 2005). Changes in soil fertility may also alter the seasonal dynamics of storage and remobilization of C and N in forest trees and play an important role in the long-term responses to ele-

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vated [CO_2].

Seasonal dynamics of C (Barbaroux et al. 2003) and N (Cheng and Fuchigami 2002, Gomez and Faurobert 2002) reserves have usually been studied separately, although C and N have interactive effects on vegetative development (Sauter and van Cleve 1994, Bollmark et al. 1999, Dyckmans et Flessa 2001, Cerasoli et al. 2004). Furthermore, there have been few studies on the dynamics of C and N reserves in forest trees in response to climate change.

Our aim was to examine the effects of N availability on storage and remobilization of C and N in *Quercus robur* saplings grown for 2 years in elevated [CO_2]. Saplings were subjected to long-term dual labeling with ^{13}C and ^{15}N isotopes at the end of the first growing season, allowing quantification of the effects of N availability and elevated [CO_2] on the accumulation of C and N compounds during the autumn, and their remobilization in spring.

Materials and methods

Plant material and cultivation

Two hundred acorns (*Quercus robur* L., Heuilly sur Saône, NE France) were sown in 5-l plastic containers filled with vermiculite. Half of the containers were placed in a 50-µm-thick transparent polypropylene tunnel (5 × 3 × 2.3 m) and exposed continuously to ambient atmospheric [CO_2] (A, 390 ± 30 µmol mol^{-1}). The other containers were placed in an identical tunnel, but exposed to elevated atmospheric [CO_2] (E, 700 ± 50 µmol mol^{-1}). The tunnels (A, E) were located in a greenhouse at INRA-Nancy, France. Seedlings in the A and E treatments were subdivided into two groups that were watered to field capacity twice daily with a complete nutrient solution (Le Blevennec 1986) containing either 6.1 (High N: HN) or 0.61 mmol N l^{-1} (Low N: LN). The seedlings were assigned to the N treatments in a full factorial design (A LN, A HN, E LN, E HN). To reduce the effect of environmental heterogeneity, plants were randomized within the tunnels throughout the experiment. Atmospheric [CO_2] in each tunnel was continuously monitored by infrared gas analyzers (ADC 225 MK3, U.K.) and controlled by an automated regulation system (Vivin et al. 1995, Maillard et al. 2001). Photosynthetic photon flux in the tunnels was about 60% of full sunlight and never exceeded 1400 µmol m^{-2} s^{-1}. Air temperature ranged from 15 to 25 °C and was maintained above 8 °C during cold periods. Vapor pressure deficit ranged from 1.01 to 2.02 kPa.

^{13}C and ^{15}N labeling

Ten saplings were randomly selected from each treatment and labeled during three 2-day cycles over one month [from October to November (Year 1)] with $^{13}CO_2$ and $^{15}NH_4NO_3$. The selected saplings were placed in a VTPH 5/1000 controlled environment chamber (Vötsch Industrietechnik GmbH, Reiskirchen-Lindenstruth, Germany) operated as a semi-closed system. The following day, each plant container was supplied with 20 ml of 10 mM $^{15}NH_4NO_3$ solution (Eurisotop, Gif sur Yvette, France). Saplings were exposed for two consecutive days to $^{13}CO_2$-enriched air (4.4 atom% ^{13}C) with a [CO_2] of either 390 or 700 µmol mol^{-1}. Chamber temperature was 20 ± 1 °C and relative humidity was 97%. Three high-pressure sodium vapor discharge lamps (SONT, Philips Electronics N.V., Amsterdam) provided a photosynthetic photon flux of 350 µmol m^{-2} s^{-1} at plant level. After each labeling cycle, labeled plants were returned to their respective tunnel.

At the end of the labeling period, roots of labeled saplings were washed to eliminate unabsorbed ^{15}N. Roots of unlabeled saplings were washed in the same way. All saplings were repotted before winter (Year 1) in 7-l plastic containers to prevent root confinement, and returned to their respective tunnel.

Sampling and growth measurements

For each treatment, half of the labeled saplings were harvested during dormancy (January of Year 2) and the remaining saplings were harvested in April of Year 2 when leaves of the new shoots had reached maturity. On each sampling date, three unlabeled saplings were harvested to assess baseline abundance of ^{13}C and ^{15}N. Harvested saplings were separated into old stem, large (diameter > 2 mm) and small (diameter < 2 mm) roots, and in April only, new leaves and new stems. Roots were washed to remove vermiculite and nutrient solution. In April, numbers and lengths of new shoots were measured. Leaf area was measured with a planimeter (Delta-T Devices, Cambridge, U.K.). To assess leaf N retranslocation efficiency, mature leaves and litter of eight unlabeled saplings were harvested in November (Year 1) and in January (Year 2), respectively. Plant material was frozen in liquid nitrogen, freeze-dried, weighed and ground to a fine powder with a laboratory mill (Tecator, Cyclotec 1093, Höganäs, Sweden) for C and N analyses.

Isotopic analyses

Total C and N contents and concentrations, and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios of plant tissues were measured with an elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled to a Delta-S isotopic ratio mass spectrometer (Finnigan–Mat, Thermoquest Corp., San Jose, CA).

Isotopic abundance (atom% or *A*%) for C and N was computed as:

$$A_C = \frac{^{13}C}{^{12}C + ^{13}C} 100$$

$$A_N = \frac{^{15}N}{^{14}N + ^{15}N} 100$$

The contributions of C and N assimilated during spring growth (Year 2) and C and N remobilized from perennial organs were calculated with equations of isotopic dilution (Deléens et al. 1994). For simplicity, only variables related to C are shown. The corresponding variables for N can be defined by substituting ^{13}C and ^{12}C with ^{15}N and ^{14}N, respectively. Atom excess % is defined as the difference between the isotopic abundance of a given plant organ following administration of the ^{13}C or ^{15}N tracers ($A_{C,lab}$%), and the ^{13}C or ^{15}N natural

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abundance measured for the same component of an unlabeled plant ($A_{C,unlab}$%).

$$^{13}C_{excess}\% = 100(A_{C,lab}\% - A_{C,unlab}\%)$$

Isotopic abundance (A%) of C in each plant organ between January (dormancy period, Year 2) to April (maturity of new spring leaves, Year 2) depended on both the relative contribution of C derived from labeled reserves (X_C, old C) of dormant plants and C assimilated during new leaf expansion (Y_C, new C) with $X_C + Y_C = 1$:

$$A_{C,lab,org}\%(X_C + Y_C) = X_C(A_{C,lab,pl}\%|_{Jan}) + Y_C(A_{C,unlab,org}\%)$$

where X_C, the proportion of old C, was estimated for a given plant as:

$$X_C = \frac{A_{C,lab,org}\%|_{Apr} - A_{C,unlab,org}\%}{A_{C,lab,pl}\%|_{Jan} - A_{C,unlab,org}\%}$$

Isotopic abundance for ^{13}C or in labeled plants ($A_{C,lab,pl}$) in January is assumed to correspond to the amount of C available for remobilization. We did not assess such values but assumed that they were equal to those of bulk plant material (Pellicer et al. 2000, Maillard et al. 2004).

Total C content (C_{tot}) comprised old C (C_{old}, incorporated before January) and new C (C_{new}, incorporated between January and April). Old C was incorporated in perennial organs of the labeled plant, and C_{new} was incorporated in newly formed leaves of the labeled plant. Thus, $C_{tot} = C_{old} + C_{new}$, where $C_{old} = X_C C_{tot}$ and $C_{new} = (1 - X_C)C_{tot}$.

Retranslocation efficiency from senescing leaves

Autumnal N retranslocation efficiency (RE_N, %) from senescing leaves was calculated according to Wang et al. (2003):

$$RE_N = \frac{N_{old}|_{Nov} - N_{litter}|_{Jan}}{N_{old}|_{Nov}} 100$$

where N_{old} is N content in old leaves, and N_{litter} is N content in litter.

Carbon and nitrogen remobilization

In perennial organs, the difference between total C (or N) content (C_{org}) in January and old C (or N) content ($C_{old,org}$) in April indicated the amount of stored C (or N) mobilized between January and April. Remobilization of C (RM_C, %) and N (RM_N, %) were calculated as:

$$RM_{C(or\ N)} = \frac{C(\text{or}\,N)_{org}|_{Jan} - C(\text{or}\,N)_{old,org}|_{Apr}}{C(\text{or}\,N)_{org}|_{Jan}} 100$$

Near-infrared spectroscopic measurements

Concentrations of soluble sugars, starch, total free amino acids, total soluble proteins, C and N of all samples of organs with a total dry mass of more than 1 g, and for each date, were determined by near-infrared spectroscopy (NIRSystems 6500, Foss NIRSystems, Raamsdonksveer, The Netherlands) as described by Joffre et al. (1992). Each sample was milled to a fine powder (Cyclotec 1093 Sample Mill, Tecator, Höganäs, Sweden) and scanned by NIRS.

Three distinct populations of NIR spectra, corresponding to leaves, stems and roots, were considered. Independent calibration equations between spectral and chemical data were built for each population with a partial least squares (PLS) algorithm (Martens and Naes 1989, Shenk and Westerhaus 1991*a*). About 60 samples were selected per population, based on Mahalanobis distances between all pairs of spectra (Select procedure of ISI software, see Shenk and Westerhaus 1991*b*), and analyzed for soluble sugars, starch, total free amino acids, total soluble proteins, carbon and nitrogen as described below. Chemical concentrations of all samples were obtained with standard errors for leaves, stems and roots, respectively, of 0.59, 0.45 and 0.75% for soluble sugars; 0.61, 0.70 and 0.70% for starch; 0.32, 1.07 and 1.07% for total free amino acids; 1.68, 1.12 and 1.12% for total soluble proteins; 0.41, 0.72 and 0.66% for carbon; and 0.10, 0.05 and 0.04% for nitrogen.

Carbohydrate analysis for NIRS calibration

Soluble sugars were extracted twice from 5–10 mg of ground sample by incubating in 80% boiling ethanol (1 ml) for 30 min and centrifuging for 10 min at 12,620 *g*. The pellet was retained, and the supernatants were combined and dried overnight in a vacuum-evaporator to eliminate ethanol. Dried extracts were solubilized in 0.5 ml of 0.02 *N* NaOH, and the fructose and sucrose converted to glucose equivalents (Boehringer 1984). Total glucose was quantified at 340 nm by an enzymatic method (hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49; Boehringer 1984). Starch was extracted from the pellet after extraction of soluble sugars by boiling for 1 h in 0.02 N NaOH, and hydrolyzed to glucose with α-amyloglucosidase (EC 3.2.1.3, Boehringer Manheim Biochemicals, Mannheim, Germany) in 0.32 M citrate buffer, pH 4.2 at 48 °C for 30 min. Glucose was assessed as described by Boehringer (1984).

Total soluble protein analysis for NIRS calibration

Total soluble proteins were extracted from 30 mg of ground tissue of each organ at 4 °C in 4 ml of 0.1 M phosphate buffer (pH 7.38) containing 60% (w/w) polyethylene glycol (PEG 20,000), polyvinyl-pyrrolidone (PVP) and 5 mM dithiothreitol. Concentrations of PVP varied with plant organ: 7% (w/w) PVP 40,000 for stems and large and small roots, and 3.75% (w/w) PVP 40,000 for leaves. Samples were sonicated (Bransonic 2510, Branson Ultrasonic Corporation, USA) for 30 min and centrifuged for 15 min at 12,620 *g*. Each supernatant was kept and the extraction was repeated twice on the pellet. The three supernatants were combined and total soluble protein concentration assessed colorimetrically at 595 nm according to Bradford (1976), using Coomassie Blue G 250

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(Bio-Rad, 500-0006), with bovine serum albumin as the standard. Assuming that proteins contain about 22.6% N (Yeoh and Wee 1994), results were expressed as mg soluble protein N $(100\ g_{DM})^{-1}$.

Total amino acid analysis for NIRS calibration

Free amino acids were extracted from 10 mg of ground tissue for 10 min at 4 °C in 650 µl of 7:3 (v/v) methanol:water (MW). The mixture was centrifuged for 5 min at 12,620 *g*. The supernatant was retained, and the pellet was rinsed twice with 2 ml of MW and collected each time by centrifuging at 12,620 *g* for 15 min. The supernatants were combined and the free amino acid concentration was determined colorimetrically at 570 nm by the ninhydrin method (Yemm and Cocking 1955), with leucine as the standard. Assuming that mean N concentration of free amino acids is 13%, results were expressed as mg amino acid N $(100\ g_{DM})^{-1}$.

Statistical analysis

The experimental design was a full factorial with two factors: $[CO_2]$ (ambient and elevated) and N fertilization (LN and HN). Treatment effects were assessed by a two-way analysis of variance (ANOVA) followed by Tukey's test.

Results

Nitrogen mobilization at leaf fall

By November of Year 1, HN increased total leaf N content 400%, whereas $[CO_2]$ had no effect on leaf N content (Figure 1a). Leaf N concentration was reduced by both elevated $[CO_2]$ and LN (Figure 1b), but there was no $[CO_2] \times N$ interaction. Leaf N represented about 30% of sapling N in all treatments (Figure 1c).

In January, litter N content, representing N lost during leaf fall, was higher in the HN treatment than in the LN treatment. Nitrogen retranslocation efficiency from senescing leaves was higher in LN saplings (60%) than in HN saplings (35%) (Figure 1a). However, in all treatments, N lost in litter equaled about 15% of sapling N (Figure 1c).

Carbon and nitrogen storage and remobilization

Accumulations of C and N were strongly enhanced by HN, with elevated $[CO_2]$ having an additive effect (Figures 2a and 3a) in all perennial organs (Figures 2b, 2d, 2f, 3b, 3d and 3f). Total C and N contents of saplings (Figures 2a and 3a) and total C content of perennial organs did not change significantly from January to April (Figures 2b, 2d, and 2e). In contrast, total N content of old stems and large roots decreased between January and April (Figures 3b and 3d) but remained constant in fine roots (Figure 3f). In April, about 15% of total C and 40% of total N were allocated to new leaves (Figures 2e and 3e).

Old C was mobilized rapidly between January and April in perennial organs, especially of E HN saplings (Figure 2b and 2d). In old stems, remobilization accounted for 50–58% of C reserves, and up to 75% in old stems of E HN saplings. In large roots of HN saplings, 55–68% of the old C was mobilized. Old N was mobilized strongly in perennial organs and at a similar rate in all treatments (Figures 3b and 3d). Nitrogen remobilization accounted for 65–79% in old stems, and for 56–69% in large roots.

Perennial organs of HN saplings had high N concentrations (Table 1). Nitrogen concentrations of old stems and large roots decreased in response to elevated $[CO_2]$ with a $[CO_2] \times N$ interaction for large roots (Table 1). Nitrogen concentrations of old stems and large roots declined sharply from January to April (Table 1). Elevated $[CO_2]$ significantly reduced N concentrations in leaves and new stems of LN saplings (Table 1).

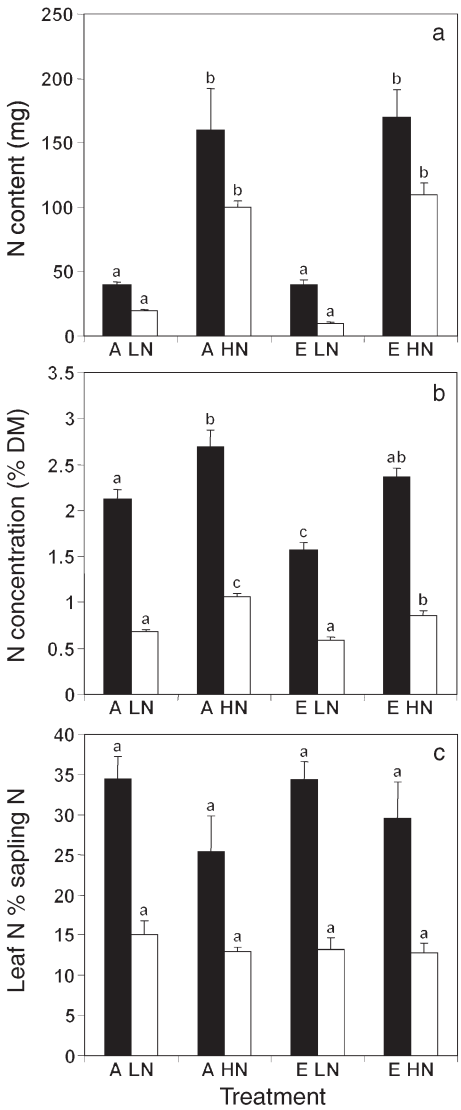

Figure 1. Nitrogen budget of old leaves (sampled in November, Year 1, filled bars) and litter (sampled after leaf fall in January, Year 2, open bars) of *Quercus robur* saplings grown in ambient (A) or elevated (E) $[CO_2]$ with low (LN) or high (HN) nitrogen fertilization. Significant treatment effects and their interactions are indicated by different letters (two-way ANOVA followed by Tukey's test, $P < 0.05$). Values are means ± SE ($n = 8$).

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[CO_2] and N supply and starch and soluble sugar storage and remobilization

In January, starch concentration was greatest in large roots (35–50% DM), followed by small roots (10–20%) and old stem (10%) (Table 2). Elevated [CO_2] had no effect on starch concentrations of perennial organs, whereas LN increased the starch concentration in large roots (A: +17%, E: +31%), decreased it in small roots (A: –35%, E: –43%) and had no effect on starch concentration in old stems (Table 2). Soluble sugar concentrations were similar in all treatments, except in old stems of E LN saplings which had increased soluble sugar concentrations (Table 2).

From January to April, starch concentrations decreased in large and small roots in all treatments, and in old stems of HN saplings (Table 2). Soluble sugar concentrations decreased in old stems of LN saplings and strongly increased in large roots of HN saplings (Table 2). In April, starch concentration of new leaves was markedly increased by both elevated [CO_2] and LN (Table 2), and a similar but less pronounced effect was observed in new stems. The HN treatment increased the proportion of soluble sugars in new organs with no [CO_2] effect (Table 2).

[CO_2] and N supply and N storage and remobilization

Soluble protein and amino acid N concentrations represented less than 0.25% of organ dry mass and about 20 and 13% of their total N, respectively (Table 1). In January, HN greatly increased soluble protein and amino acid N concentrations. Elevated [CO_2] decreased soluble protein N (on average 17%) and amino acid N (37%) concentrations in HN saplings but had no effect in LN saplings (Table 1).

From January to April, soluble protein N concentrations decreased significantly in old stems in all treatments. Amino acid N concentrations were decreased in both old stems and large roots in all treatments (Table 1). In April, soluble protein N concentration in new leaves was higher than in perennial organs. The HN treatments greatly increased soluble protein and amino acid N concentrations in new organs, and this effect was lessened by elevated [CO_2] (Table 1).

Carbon dioxide concentration, nitrogen supply and spring growth

Spring growth was markedly increased by HN leading to large changes in development and organogenesis of axillary organs (Table 3). Lengths of new axes and numbers of branches and leaves were significantly increased in HN saplings compared with LN saplings (Table 3). In contrast, the N treatments had no effect on specific leaf area (SLA). Sapling morphology was modified by elevated [CO_2] (Table 3): branch length, main leaf area and SLA were markedly decreased, whereas numbers of

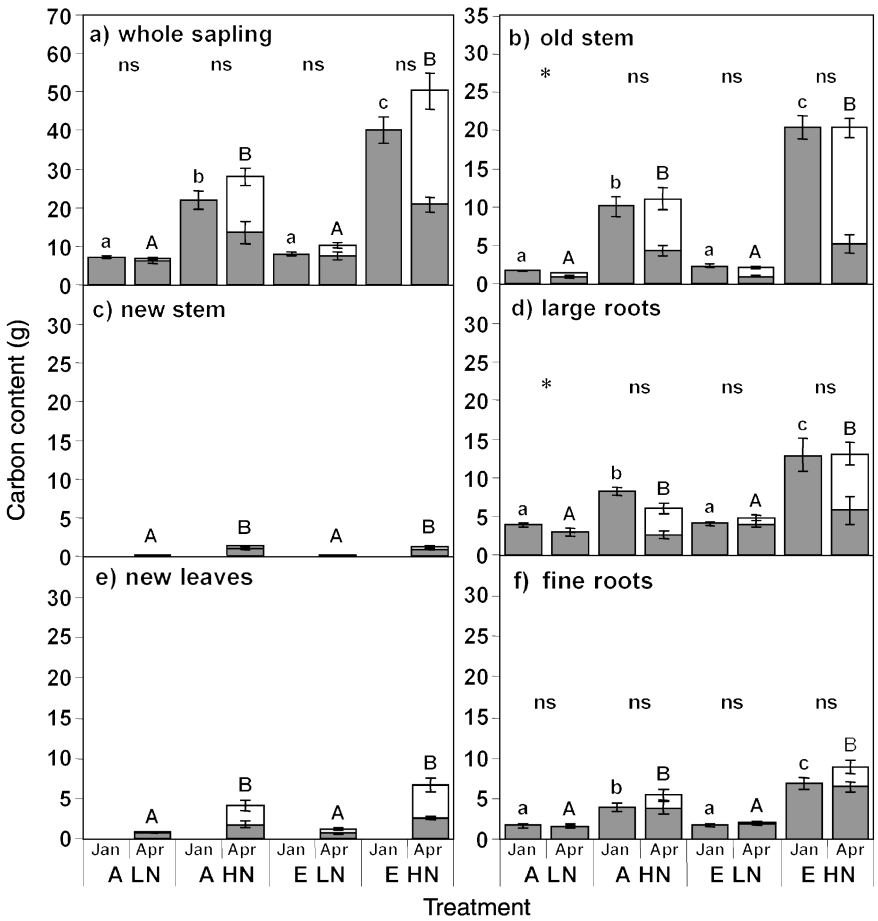

Figure 2. Old (filled bars) and new (open bars) C contents in whole saplings and organs of *Quercus robur* grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization, sampled in winter (January, Year 2) and after spring shoot flush (April, Year 2). Means ± SE (n = 8). For each organ, the significance of treatment effects and their interaction on total C are indicated by different letters (lowercase for January; uppercase for April) (two-way ANOVA followed by the Tukey test, $P < 0.05$). For whole saplings (a) and perennial organs (b, d, f), significant differences in total C content between January and April are indicated at the top of each figure (ns, non significant; *, $P < 0.05$).

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branches and axillary leaves were strongly increased. The root:shoot ratio was strongly increased by LN, particularly in the ambient [CO_2] treatment (Table 3).

[CO_2] and N supply and partitioning of newly assimilated C and N between new and old organs

In April, new C and N uptake in whole saplings depended more on N availability than on [CO_2] (Figures 2a and 3a). At the end of leaf expansion, 50–60% of the C in HN saplings originated from assimilation compared with only 10–20% in LN saplings (Figure 2a). New N was incorporated in HN saplings only and represented less than 20% of total N (Figure 3a). New leaves contained 50–60% of new C, except in A LN saplings, where new C represented only 15% of total C (Figure 2e). The fraction of new C in new stems was low and never exceeded 20% of total C (Figure 2c) in HN saplings. New organs were built mostly with old N (75–95% of total N) (Figures 3c and 3e). New C and N were found in perennial organs (Figures 2b, 2d, 2f, 3b, 3d and 3f), with the largest accumulation occurring in old stems with about 45% of total new C and 33–49% of new N (Figures 2b and 3b).

Discussion

Elevated [CO_2] and N fertilization increased second-year growth of *Quercus robur* saplings, with N treatment having the greatest effect. This finding is in agreement with earlier results for *Quercus virginiana*, *Quercus ilex* (Tognetti and Johnson 1999*a*, 1999*b*) and *Quercus robur* (Maillard et al. 2001). Elevated [CO_2] induced similar changes in several other tree species (Saxe et al. 1998), but the physiological mechanisms controlling these changes remain poorly understood. Cell division and cell expansion may be affected, driven mainly by increased substrate availability and by differential expression of genes involved in cell cycling or cell expansion mediated by plant hormones (Pritchard et al. 1999). Low N supply typically induces a higher root:shoot ratio, an adjustment in biomass allocation in response to an imbalance of exogenous resources that tends to increase the supply of the limiting resource (Marschner 1995, Hermans et al. 2006).

Although elevated [CO_2] and HN stimulated growth of new shoots (Table 3) at the sapling level (Figures 2a and 3a), we observed no differences in total C and N contents between winter and spring, indicating that new C assimilation and N uptake were not achieved or were compensated by respiratory losses, and that initial new growth measured in April depended on C and N stored in perennial organs. We hypothesized that elevated [CO_2] and N fertilization increased the storage of both C and N compounds during the previous year and their remobilization at the beginning of the following spring.

In January, all perennial organs, especially those of HN saplings, had accumulated more C (Figures 2b, 2d and 2e) and more N (Figures 3b, 3d and 3e) in the elevated [CO_2] treatment than in the ambient [CO_2] treatment. The higher C:N ratio of saplings in the elevated [CO_2] treatment than in the ambient

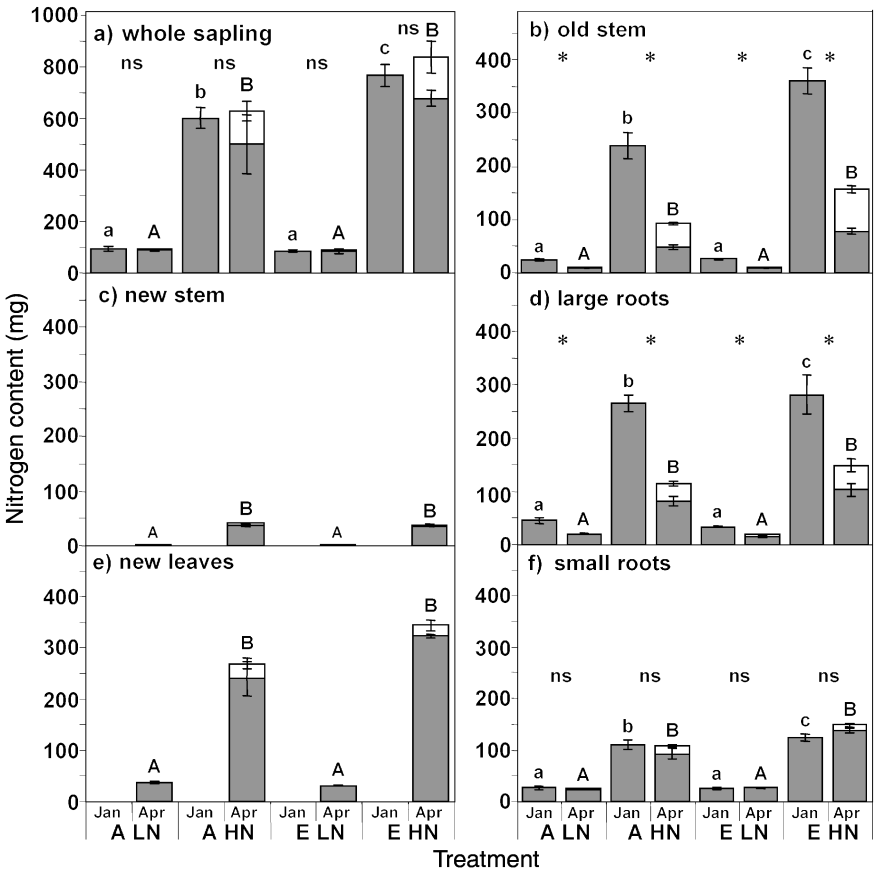

Figure 3. Old (filled bars) and new (open bars) nitrogen contents in whole saplings and individual organs of *Quercus robur* grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization, sampled in winter (January, Year 2) and after the new spring flush was expanded (April, Year 2). Means ± SE (n = 8). For each organ, the significance of treatment effects and their interaction on total N are indicated by different letters (lowercase for January, uppercase for April) (two-way ANOVA followed by the Tukey test, $P < 0.05$). For whole saplings (a) and perennial organs (b, d, f), significant differences in total N content between January and April are indicated at the top of each figure (ns, non significant; *, $P < 0.05$).

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[CO_2] treatment indicated that the additional increase in C was higher than the increase in N. Maillard et al. (2001) reported that, in 1-year-old oak saplings, elevated [CO_2] and HN increased net CO_2 assimilation more than ^{15}N uptake, creating a C:N ratio imbalance in summer that was maintained after leaf shedding. Moreover, during leaf shedding the previous year, HN saplings recovered N from senescing leaves less efficiently than LN saplings (Figure 1a). Similar behavior was observed in N-deficient *Malus domestica* Borkh. and *Fagus sylvatica* L. (Millard and Thomson 1989, Dyckmans and Flessa 2001). These findings led us to hypothesize that N recovery from old leaves is more essential for survival and spring growth in N-deficient trees than in well-fertilized trees.

In January, oak saplings stored C as starch mainly in large roots, and starch concentrations were higher in LN saplings than in HN saplings with no effect of elevated [CO_2] (Table 2). Nitrate limitation typically leads to large increases in starch concentration in plants (Stitt and Krapp 1999, Cheng and Fuchigami 2002). It has been assumed that starch accumulation is a passive response to decreased rates of growth. Conversely, in well-fertilized oak saplings, lower starch concentrations, even under elevated [CO_2] conditions, imply that structural compounds account for a larger proportion of total biomass. Reduced starch accumulation could be related both to increased carbon requirements for the assimilation of inorganic N and to rapid consumption for the synthesis of structural compounds that occurred during the first year, as also shown in *Pinus nigra* L. (Luo et al. 2006).

In January, elevated [CO_2] decreased N concentrations in all perennial organs only in the HN saplings (Table 1). This effect has been interpreted in different ways: higher nitrogen-use efficiency, higher growth under an elevated [CO_2], accelerated senescence, and inadequate N fertilization or N uptake (Stitt and Krapp 1999). Nitrogen was stored in both soluble proteins and amino acids in all perennial organs. The HN treatment typically increased soluble protein and amino acid N storage in perennial organs (Table 1), but this effect was reduced by elevated [CO_2]. The HN-induced increase in amino acid N concentration was greater than the increase in soluble protein N concentration with the result that the ratio of soluble protein N to amino acid N was reduced by fertilization. Preferential storage of additional N in free amino acids rather than proteins in

Table 1. Nitrogen (N), soluble protein and amino acid N concentrations (g (100 g_{DM})$^{-1}$) in organs of *Quercus robur* saplings grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertiliation. Saplings (n = 8) were sampled in winter (January, Year 2) and after the new spring flush had expanded (April, Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different lowercase letters indicate significant differences between treatments (two-way ANOVA, $P < 0.05$). Different uppercase letters indicate significant differences between January and April within a treatment.

Organ	Month	Treatment				Significance of treatment effects		
		A LN	A HN	E LN	E HN	[CO_2]	N	[CO_2] × N
Nitrogen concentration (% DM)								
Old stem	January	0.68 ab B	1.12 c B	0.56 a B	0.82 b B	***	***	ns
	April	0.38 b A	0.48 c A	0.27 a A	0.39 b A	***	***	ns
Large roots	January	0.50 a B	1.40 c B	0.38 a B	0.98 b B	***	***	*
	April	0.32 ab A	0.99 c A	0.21 a A	0.60 b A	**	***	ns
Small roots	January	0.78 a A	1.31 b A	0.74 a A	0.86 a A	ns	***	ns
	April	0.75 a A	1.11 b A	0.66 a A	0.86 a A	**	***	ns
New leaves	April	2.08 b	3.17 d	1.24 a	2.49 c	***	***	ns
New stems	April	0.86 b	1.41 c	0.63 a	1.21 c	**	***	ns
Soluble protein nitrogen concentration (% DM)								
Old stem	January	0.16 a B	0.20 b B	0.14 a B	0.17 a B	**	***	ns
	April	0.10 a A	0.12 b A	0.08 a A	0.10 a A	***	***	ns
Large roots	January	0.11 a A	0.21 c B	0.09 a B	0.16 b A	***	***	*
	April	0.09 ab A	0.17 c A	0.05 a A	0.12 b A	***	***	ns
Small roots	January	0.18 a A	0.26 c A	0.18 a A	0.22 b A	**	***	*
	April	0.17 a A	0.25 b A	0.17 a A	0.20 a A	*	***	**
New leaves	April	0.36 b	0.55 d	0.28 a	0.46 c	***	***	ns
New stems	April	0.18 a	0.28 b	0.16 a	0.27 b	ns	***	ns
Amino acid nitrogen concentration (% DM)								
Old stem	January	0.07 a B	0.15 c B	0.06 a B	0.11 b B	**	***	*
	April	0.02 a A	0.04 b A	0.01 a A	0.02 a A	**	***	ns
Large roots	January	0.10 a B	0.33 c B	0.08 a B	0.23 b B	**	***	*
	April	0.05 ab A	0.21 c A	0.01 a A	0.13 b A	**	***	ns
Small roots	January	0.04 a A	0.15 b B	0.04 a A	0.07 a A	***	***	***
	April	0.05 ab B	0.08 b A	0.02 a A	0.05 ab A	**	**	ns
New leaves	April	0.03 b	0.04 c	0.02 a	0.03 b	***	***	ns
New stems	April	0.03 a	0.12 b	0.02 a	0.09 b	*	***	ns

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perennial organs was also shown in apple trees (Cheng et al. 2004). This mechanism provides saplings the advantage that amino acids are readily available when growth resumes in spring (Cheng et al. 2004). Elevated [CO_2] decreased both soluble N concentration and total N concentration in fertilized oak saplings.

By using ^{15}N- and ^{13}C-labeling of reserves before winter, we demonstrated that spring growth of *Quercus robur* was strongly controlled by the availability of internal resources. About 20% of old C (Figures 2c and 2e) and 50% of old N

Table 2. Starch and soluble sugar concentrations (g 100 $g_{DM})^{-1}$) in organs of *Quercus robur* saplings grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization. Saplings ($n = 8$) were sampled in winter (January; Year 2) and after the new spring flush had expanded. (April; Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different lowercase letters indicate significant differences between treatments (two-way ANOVA, $P < 0.05$). Different uppercase letters indicate significant differences between January and April within a treatment.

Organ	Month	Treatment				Significance of treatment effects		
		A LN	A HN	E LN	E HN	[CO_2]	N	[CO_2] × N
Starch concentration (% DM)								
Old stem	January	8.10 a A	8.21 a B	8.22 a A	7.31 a B	ns	ns	ns
	April	8.86 b A	1.96 a A	9.87 b A	2.09 a A	ns	***	ns
Large roots	January	46.29 b B	39.59 a B	46.32 b B	35.19 a B	ns	***	ns
	April	29.19 b A	8.70 a A	37.92 b A	15.13 a A	**	***	ns
Small roots	January	12.42 a B	18.92 b B	11.68 a B	20.36 b B	ns	***	ns
	April	4.97 ab A	1.94 a A	5.10 ab A	6.00 b A	*	ns	*
New leaves	April	5.92 b	1.23 a	15.84 c	5.01 ab	***	***	*
New stems	April	4.75 b	1.97 a	8.27 c	1.88 a	*	***	*
Soluble sugar concentration (% DM)								
Old stem	January	3.33 ab B	3.14 a A	3.87 b B	3.29 ab A	ns	*	ns
	April	1.93 a A	4.06 b A	1.97 a A	3.79 b A	ns	***	ns
Large roots	January	3.48 a A	3.07 a A	3.82 a A	3.03 a A	ns	ns	ns
	April	6.22 b A	9.01 c B	4.54 a A	7.08 b B	***	***	ns
Small roots	January	2.88 a A	3.17 a A	3.13 a A	3.36 a A	ns	ns	ns
	April	2.48 a A	4.91 b B	2.64 a A	4.00 b A	ns	***	ns
New leaves	April	3.48 a	7.71 b	3.91 a	7.29 b	ns	***	ns
New stems	April	2.23 a	5.60 b	2.36 a	5.87 b	ns	***	ns

Table 3. Growth variables of new shoots and biomass root: shoot ratio of *Quercus robur* saplings grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization. Saplings ($n = 8$) were sampled after the new spring flush had expanded (April, Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different letters indicate significant differences between treatments (two-way ANOVA followed by the Tukey test, $P < 0.05$).

Organ	Treatment				Significance of treatment effects		
	A LN	A HN	E LN	E HN	[CO_2]	N	[CO_2] × N
Length (cm)							
Main stem	11.90 a	23.40 b	10.00 a	14.90 ab	ns	**	ns
Branches	8.10 a	13.10 b	6.20 a	9.90 ab	*	**	ns
Number							
Branches	5.60 a	21.70 b	8.10 a	32.80 c	**	***	ns
Main leaves	9.10 a	12.80 a	8.90 a	11.90 a	ns	*	ns
Axillary leaves	19.50 a	200.20 b	42.40 a	354.10 c	**	***	*
Area (dm^2)							
Main leaves	3.91 ab	5.08 b	2.02 a	2.32 ab	**	ns	ns
Axillary leaves	2.72 a	34.82 b	4.19 a	32.55 b	ns	***	ns
Specific leaf area (dm^2 g^{-1})							
Main leaves	2.63 b	2.41 b	1.98 a	2.22 ab	***	ns	*
Axillary leaves	3.16 c	2.73 b	2.16 a	2.49 ab	***	ns	***
Root:shoot ratio	3.87 c	1.31 a	2.91 b	1.12 a	***	***	*

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(Figures 3c and 3e) were used in oak saplings for shoot growth in spring.

Carbon of new stems originated almost entirely from internal C resources (Figure 2c). In contrast, new leaves comprised 60% new C (Figure 2e), this percentage being smaller in LN saplings than in HN saplings. Conversely, Dyckmans and Flessa (2001) reported that, 2 weeks after bud burst, 38.9 and 52.7% of leaf C in HN- and LN-pretreated 3-year-old beech trees was derived from new C. However, in our experiment, the total amount of new C in beech leaves was 43% lower in LN trees compared with HN trees, 12 weeks after bud burst. Elevated [CO_2] significantly modified branching architecture (i.e., leaf and branch numbers, branch length) of HN saplings (Table 3), resulting in increased leaf area and subsequent C acquisition in an expected positive feedback loop. In fully expanded leaves of E HN saplings, the large amount of new C, which was unaccompanied by starch accumulation (Table 2), may indicate efficient utilization of new C for structural syntheses as reported in leaves of northern red oak seedlings (Dickson et al. 2000).

Remobilization of internal C from perennial organs was increased by HN, and additional remobilization from old stems was observed in response to elevated [CO_2] (Figure 2). For HN saplings, not all old C mobilized from the perennial organs was allocated to new shoots (Figure 2), reflecting respiratory C losses during the winter (maintenance) and early spring (growth). Respiratory C loss was compensated by new assimilation because the total C content did not change between January and April (Figures 2b, 2d and 2f). Thus, HN saplings renewed 50–60% of their total C during April (Figure 2a).

Because allocation of new C occurred in perennial organs and we did not assess ^{13}C labeling of starch, we were unable to calculate remobilization (old C) and renewal (new C) in the total starch pool. However, differences in starch concentrations were observed between January and April, suggesting that starch remobilization occurred in most perennial organs (Table 2). Spring remobilization of starch often results in its depletion in perennial organs, and the lowest starch concentrations have been observed just before bud break in some deciduous trees (Witt and Sauter 1994, Piispanen and Saranpää 2001, Barbaroux et al. 2003). The extent of starch remobilization was higher in HN saplings than in LN saplings (Table 2). The decrease in starch concentration in HN saplings was associated with an accumulation of soluble sugars in large roots and no change in old stems. We hypothesized that, in old stems of HN saplings, a significant portion of the soluble carbohydrates formed during starch hydrolysis is promptly converted to structural carbon compounds or exported. In contrast, in the old stems of LN saplings, no starch remobilization occurred in April, resulting in greater starch accumulation compared with HN saplings, which was not observed in January.

Our labeling experiment confirmed that, until April, no major N absorption occurred to supply N for new spring growth (Figure 3). Nitrogen uptake occurred only in HN saplings, and some new N was allocated to new shoots and new leaves (15–20% of total new N). During this period, internal N stores were used for new growth has been shown in *Fagus sylvatica* L. (Dyckmans and Flessa 2002) and *Alnus glutinosa* (L.) Gaertn., despite the latter species being an N_2-fixer (Temperton et al. 2003). In oak saplings, the extent of N remobilization was unaffected by elevated [CO_2] (Figure 3) (cf. Maillard et al. 2001). In contrast, Temperton et al. (2003) reported that elevated [CO_2] increased N remobilization for leaf growth in *A. glutinosa* but not in *Pinus sylvestris* L. When leaves had just completed expansion, N concentrations of all organs were lower in E HN saplings than in A HN saplings (Table 1). This difference may be partly linked to a delay between new CO_2 assimilation and new N uptake, because the new C:new N ratio was higher in E HN saplings than in A HN saplings (Figure 3). Nitrogen remobilization from perennial organs was not compensated by new N accumulation, leading to decreased N content over time (Figures 3b and 3d). Several studies have reported that stored N is mobilized and used to support early shoot growth leading to a depletion of stored N in source organs such as branches (Millard 1996, Sauter and Wellenkamp 1998). Under natural conditions, replenishment of N reserves in perennial organs mainly occurs at the beginning of leaf yellowing during late summer and early fall (Sauter and Witt 1997), so that until this time, N uptake is necessary for further N reserve replenishment to occur. Even if soluble proteins were significantly mobilized to support spring growth (Table 1), our results show that amino acids were the most heavily mobilized N fraction, as observed in *Prunus persica* L. by Gomez and Faurobert (2002) who hypothesized that the role of storage proteins is linked to growth initiation in spring.

In conclusion, through primary effects on C and N storage compounds, elevated [CO_2] and N fertilization have indirect consequences on remobilization the following spring, which supports new growth of oak saplings. Our results demonstrated that initial spring growth was strongly controlled by the availability of internal N. The contrasting N storage capacities imposed during the first year affected both C and N remobilization and uptake the following year. New spring shoot architecture was modified by elevated [CO_2] and N fertilization, leading rapidly to more efficient C acquisition and N uptake. However, immediately following full leaf expansion, the delay between new CO_2 assimilation and N uptake resulted in a decrease in N concentrations in E HN saplings. During this period, internal cycling of N in the tree might help overcome N deficiency under elevated [CO_2] conditions. Our data demonstrate the importance of N storage and remobilization for the initial growth of oak saplings.

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Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ $^{13}CO_2$ pulse labelling of 20-year-old beech trees

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Summary The study of the fate of assimilated carbon in respiratory fluxes in the field is needed to resolve the residence and transfer times of carbon in the atmosphere–plant–soil system in forest ecosystems, but it requires high frequency measurements of the isotopic composition of evolved CO_2. We developed a closed transparent chamber to label the whole crown of a tree and a labelling system capable of delivering a 3-h pulse of 99% $^{13}CO_2$ in the field. The isotopic compositions of trunk and soil CO_2 effluxes were recorded continuously on two labelled and one control trees by a tuneable diode laser absorption spectrometer during a 2-month chase period following the late summer labelling. The lag times for trunk CO_2 effluxes are consistent with a phloem sap velocity of about 1 m h^{-1}. The isotopic composition ($\delta^{13}C$) of CO_2 efflux from the trunk was maximal 2–3 days after labelling and declined thereafter following two exponential decays with a half-life of 2–8 days for the first and a half-life of 15–16 days for the second. The isotopic composition of the soil CO_2 efflux was maximal 3–4 days after labelling and the decline was also well fitted with a sum of two exponential functions with a half-life of 3–5 days for the first exponential and a half-life of 16–18 days for the second. The amount of label recovered in CO_2 efflux was around 10–15% of the assimilated $^{13}CO_2$ for soil and 5–13% for trunks. As labelling occurred late in the growing season, substantial allocation to storage is expected.

Keywords: carbon allocation, Fagus sylvatica, residence time, soil CO_2 efflux, trunk CO_2 efflux.

Introduction

The anthropogenic perturbation of the earth carbon cycle and the associated climate changes are partly mitigated by carbon sequestration in the terrestrial biosphere, that is itself affected by the atmospheric CO_2 and the climate (Cox et al. 2000). This is a major issue for international policy on climate change and carbon budgets within the Intergovernmental panel on climate change (Houghton 2001). Among the terrestrial ecosystems, forests largely contribute to the biospheric carbon exchange and carbon stock (Saugier et al. 2001). Ecosystem respiration almost balances photosynthetic carbon assimilation in forest ecosystems, the net ecosystem exchange being one order of magnitude smaller than the two gross fluxes (Granier et al. 2000, Valentini et al. 2000). Small variations of one of these two fluxes due to climate changes could therefore strongly affect the carbon budget of the ecosystem and its ability to sequester carbon, possibly leading to positive or negative feedbacks (Valentini et al. 2000, Ciais et al. 2005).

Although ecosystem respiration includes contributions from the aerial part of trees (leaves, branches and stems), two-thirds of its sources are located on the ground and below the ground (Janssens et al. 2001). These sources include roots and associated symbiotic organisms such as mycorrhizal hyphae, rhizospheric microbes that feed on root exudates and saprotrophic bacteria and fungi that feed upon above-ground and below-ground litter such as coarse woody debris (Nadelhoffer and Raich 1992, Raich and Schlesinger 1992, Epron et al. 1999, 2001, Ngao et al.

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2005). In particular, the potential importance of the carbon flux to the rhizosphere through roots and mycorrhiza has been recognized for forest ecosystems but is poorly documented by in situ measurements (Högberg and Read 2006). A precise partitioning of these fluxes would require a better knowledge of the carbon residence time in each of the ecosystem compartments, as well as a better understanding of carbon allocation among these compartments. As Trumbore (2006) recently pointed out, carbon allocation patterns between growth, storage and respiration of different tree compartments and the soil are not yet well understood.

Stable isotopes, especially ^{13}C, are widely used in ecology as tracers in trophic webs (Dawson et al. 2002, Cerling et al. 2007). The fate of carbon in the soil plant system can be followed by pulse-labelling plants in the field with $^{13}CO_2$ for a short period of time (< 1 day). The ^{13}C assimilated by plants during the pulse labelling is then tracked in the respiratory fluxes during the following days and weeks (chase period). Up to now, this approach has been mostly used under controlled conditions, but a few in situ studies concerned herbaceous species (Ostle et al. 2000, Johnson et al. 2002, Rangel-Castro et al. 2004, Leake et al. 2006) or small tree saplings, either transplanted (Phillips and Fahey 2005) or grown in pots (Lacointe et al. 1993). Recently, Carbone et al. (2007) and Högberg et al. (2007) pulse labelled small boreal conifers growing in the field (with $^{14}CO_2$ and $^{13}CO_2$, respectively), to resolve the relative roles of new photosynthetic products as sources of below-ground and above-ground respiration.

The accurate determination of residence and transfer times of carbon in the atmosphere–plant–soil system requires frequent measurements of the isotopic composition of evolved CO_2 during the chase period following the short-term labelling with $^{13}CO_2$. Up to now, both cost and time required for analysing air samples by mass spectrometry in the laboratory limit frequency and duration of isotopic measurements in experiments studying either variations of natural isotopic abundance during seasons or changes in isotopic enrichment after a pulse labelling. The recent development of tuneable diode laser absorption spectrometers (TDLAS) allows in situ simultaneous measurements of effluxes of $^{13}CO_2$ and $^{12}CO_2$ at a high frequency. TDLAS have recently been used to examine ecosystem functioning (Bowling et al. 2003, Griffis et al. 2004, Barbour et al. 2007, Bahn et al. 2009, Marron et al. 2009), and this is a promising tool for tracking ^{13}C in respiratory fluxes after pulse labelling (Bahn et al. 2009).

The aim of this study was to develop and test a closed transparent chamber that was designed to label the whole crown of a tree and a labelling system capable of delivering a 3-h pulse of $^{13}CO_2$ in the field. The isotopic compositions of trunk and soil CO_2 effluxes were recorded continuously by TDLAS using a trace gas analyser during a 2-month chase period. The high temporal resolution enables accurate assessments of the time lag between photosynthetic assimilation by the crown and CO_2 release from the trunk and the soil, as well as the precise determination of the half-life of labelled carbon in a given respiratory efflux.

Materials and methods

Study site

The study was conducted in the state forest of Hesse (Hesse 2, 48°40′40″ N and 7°04′05″ E, 300 m elevation, Northeast of France) in September 2008. The study site is located in a mixed stand of 20-year-old beeches (*Fagus sylvatica* L., 63% of basal area, G), hornbeams (*Carpinus betulus* L., 26% of G), oaks [*Quercus petreae* (Matt.) Lielb. and *Quercus robur* L., averaging 8% of G], willows (*Salix caprea* L., 2% of G) and birches (*Betula pendula* Roth, 1% of G). This stand originated from natural regeneration (Ngao et al. 2007). The soil is classified as luvisol and is covered by mull humus. The average stand height is 10 m.

Incident photosynthetic photon flux density (PPFD) was measured above the stand at 16 m height (top of eddy covariance flux tower) with a photosynthetically active radiation (PAR) sensor (Delta T-BF2, Cambridge, UK). Air temperature and relative humidity were also monitored (Vaisala HMP45, Helsinki, Finland). Soil temperature at 10 cm depth was measured with four home-made copper-constantan thermocouples. Data acquisition was made with a datalogger (CR7, Campbell UK) at 10-s intervals, and 30 min averages were calculated and stored. The datalogger failed to store data between 27 October and 4 November. Volumetric soil water content at 15 cm depth was measured with a TDR probe (Trase, SoilMoisture Equipment Corp., Goleta, CA) at five locations in the vicinity of the eddy covariance flux tower.

In March 2008, three codominant trees were selected in a pure beech patch (8–9 m tall trees). Two of these trees were selected for labelling (LT1 and LT2) and one tree was selected as a control (CT). Trunk diameters at 1.3 m above soil level were 21, 28 and 20 cm, respectively. Suppressed trees that were in the vicinity of the selected trees were removed and 60 cm deep and 1.5 m long trenches were dug around each tree. Because of the presence of a compact clay horizon at 50 cm depth, almost no roots were found < 50 cm (Zapater and Granier, unpublished results). The trenches were lined with a polyethylene film and refilled with the displaced earth. The root system of each tree was thus confined in a soil volume below a square surface area of 3.56, 2.06 and 3.45 m^2 for LT1, LT2 and CT, respectively. All roots and root exudates within this area therefore originated from the isolated tree, and all roots and root exudates of this tree were contained in this volume. This trenching process ensured that there was no dilution of root respiration of the labelled trees by unlabelled ones.

One month after labelling (mid-October), the crowns were enclosed in a net to collect all the leaf litter. The leaf

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litter in the net was collected in mid-November and weighed just after collection and after oven drying at 60 °C for 48 h. A subsample (4–6% of fresh weight) was taken before drying and leaf area (LA) was determined, allowing us to estimate the total LA of the crown. The total LA of the crown was converted into green leaf mass by dividing LA by SLA, the specific leaf area of leaves sampled just after labelling (see below).

Whole crown labelling chamber

Two 10-m height stainless steel scaffoldings were built in parallel to each other around the tree to install the chamber at the top of the tree (Figure 1). Ropeholes, isolated from the inside chamber by a seal, were placed every 15 cm at the top of the chamber to accommodate a nylon rope that secured the top of the labelling chamber on four stainless steel bars. The bars were firmly attached to the top of the two scaffoldings. The base of the chamber was also secured on three stainless steel bars that were attached to the scaffoldings.

The top of the tree crown labelling chamber was a 6.25 m^2 square made of 200-µm-polyane film (celloflex 4TT; Prosyn polyane, Saint Chamond France), and the base of the chamber (1 m^2) was made with two stainless steel half-plates (2 mm thick) with circular openings to accommodate the trunk (92 mm diameter) and the two cooling device tubes (251 mm diameter). The two half-plates were joined using aluminium tape (Pure aluminium adhesive tape, Plasto SAS, Dijon, France) and sealed to the trunk with putty (terostat VII, Henken, Düsseldorf, Germany). The four sides of the chamber were 4.8 m height for LT1 and 7.1 m height for LT2. The first 1.3 m for LT1 and 4.8 m for LT2 were vertical, whereas the following parts of the sides were oblique to reduce the width from 2.5 m at the top to 1 m at the base (see Figure 1). According to the side dimension of each tree crown, the maximum volume of the labelling chamber was 19.5 m^3 for LT1 and 37.5 m^3 for LT2. The chamber could not be fully opened because of the surrounding trees, which means that the true chamber volumes were lower than the maximum values.

The top and the four sides of the chamber were sealed and three of the four sides were also sealed together before installation on the tree. The last side was stuck to the other after installing the chamber around the tree using 100 mm width transparent tape (Nitto PVC 21, Nitto Europe NV, Genk, Belgium). The four sides were stuck to the stainless steel base similarly. Air-tightness of the chamber was evaluated by comparing the amount of $^{13}CO_2$ delivered and the amount of ^{13}C recovered in the leaves after the labelling (see Results section).

Air temperature and air humidity inside the tree crown labelling chamber were recorded with one probe (HMP50, Vaisala, Finland) attached to the tree crown. Two PAR sensors (JYP 1000, SDEC France, Reignac sur Indre, France) were installed at the top of the tree. Data acquisition was made with a datalogger (CR1000, Campbell UK) at 30-s intervals. We failed to record the data during the first hour of the first labelling experiment, but some data were recorded manually. The chamber air was controlled and maintained at the temperature of the outside air by circulating air at a rate of 1200 m^3 h^{-1} through an air- and water-cooled condensing axial fan outdoor unit (AXCZ 221, HITECSA, Vilanova i la Geltrú, Spain) connected to an air conditioner (BSH-BZ 221, HITECSA, Vilanova i la Geltrú, Spain). The axial fan was modified

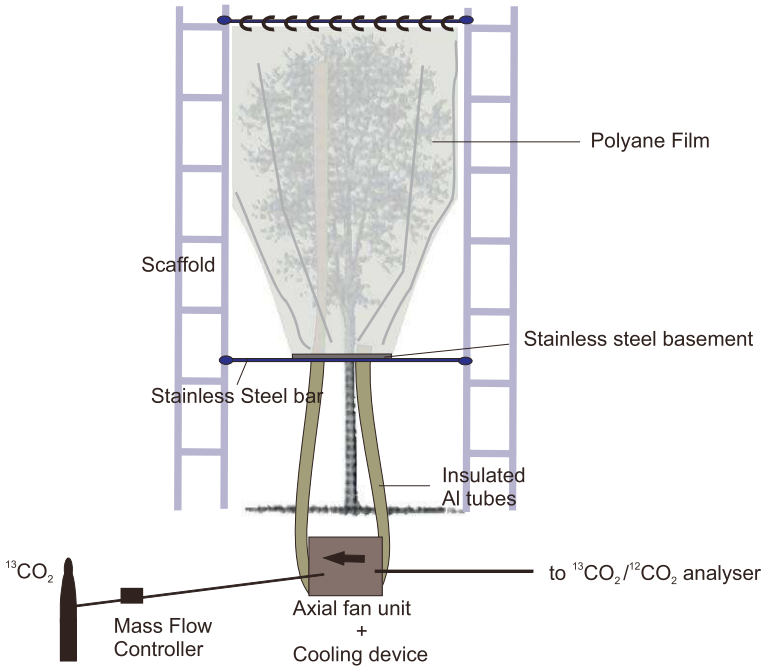

Figure 1. Diagram of the whole crown labelling chamber. Air for $^{12}CO_2$ and $^{13}CO_2$ monitoring was continuously sampled at the inlet of the cooling device. $^{13}CO_2$ was injected at the outlet of the cooling device. This figure appears in colour in the online version of *Tree Physiology*.

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(Frige Clim Zalesky, Nancy, France) to connect tubes directly to the outlet and inlet of the fan, avoiding exchange with the outside air. Air was circulating from the bottom of the chamber to the cooling device and from the cooling device to the top of the chamber through insulated aluminium tubes (250 mm diameter), which ensured good mixing of the air within the chamber. The volume of tubes and cooling device was 0.5 m^3. Including tubes and cooling device, the total volume were estimated at 11.4 and 20.1 m^3 for LT1 and LT2, respectively. We did not correct the chamber volume for the trunk and branches as they accounted for < 0.2% of the total chamber volume (see Damesin et al. 2002, for a set of allometric equations for beech trees in Hesse). Chamber air was recirculated through the cooling unit at least every minute, which was enough to ensure good temperature control and good mixing of the air.

Pulse labelling

LT1 and LT2 were labelled on 9 September (day of year 253) and on 16 September (day of year 260), respectively. The labelling chamber was closed at 10:00 UT for the first tree and at 9:30 UT for the second one. Evolution of $^{12}CO_2$ and $^{13}CO_2$ concentrations ($[^{12}CO_2]$ and $[^{13}CO_2]$, respectively) inside the chamber was monitored simultaneously with a $^{12}CO_2/^{13}CO_2$ infrared gas analyser (S710, SICK/MAIHAC, Germany). The CO_2 concentration declined after the chamber was closed because of photosynthesis (see Results section).

When $[^{12}CO_2]$ reached values < 120 μmol mol^{-1} for the first tree (10:50 UT) and 190 μmol mol^{-1} for the second tree (10:30 UT), 25 l of pure $^{13}CO_2$ (99.299 atom %, Eurisotop, Cambridge Isotope Laboratory Inc., Andover, MA) was injected at a flow rate setting between 0.11 and 0.18 l min^{-1} using a mass flow controller (Bronkhorst, Netherlands) in the air stream coming from the cooling device. We considered that the beginning of the labelling period was also the beginning of the chase period (time zero on graphs). Average $[^{13}CO_2]$ inside the chamber, measured at the outlet of the chamber, was around 425 μmol mol^{-1} ($[^{12}CO_2]$ around 90 μmol mol^{-1}) for LT1 and around 520 μmol mol^{-1} ($[^{12}CO_2]$ around 165 μmol mol^{-1}) for LT2. Abundances were 83 atom% ^{13}C (δ = 420,000‰) and 76 atom% ^{13}C (δ = 280,000‰), respectively, as compared to ambient air that is 1.1 atom% ^{13}C (δ = −8‰).

After 3- (first labelling) and 2.5-h (second labelling), the gas cylinder was emptied. The $[^{13}CO_2]$ of the chamber then declined progressively to 95 and 160 μmol mol^{-1} for LT1 and LT2 before we opened and removed the tree crown labelling chamber at 14:35 UT and 15:05 UT for LT1 and LT2, respectively. The labelling period lasted 03:45 h for LT1 and 04:35 h for LT2.

Assuming that the leak of CO_2 from the chamber to the outside air was negligible, the rate of change of $[^{13}CO_2]$ ($d[^{13}CO_2]/dt$) depends on the net rate of $^{13}CO_2$ uptake by the crown (U) and on the amount of $^{13}CO_2$ injected in the chamber (I):

$$\frac{d[^{13}CO_2]}{dt} = -U + I. \quad (1)$$

The total amount of $^{13}CO_2$ taken up by the crown was calculated by integrating U over the labelling period.

Soil and trunk CO_2 effluxes and ^{13}C composition

Soil and trunk CO_2 effluxes (F_S and F_T) and their isotopic composition ($\delta^{13}C_{FS}$ and $\delta^{13}C_{FT}$) were measured by TDLAS with a trace gas analyser (TGA 100A, Campbell Scientific, Inc., Logan, UT) coupled to flow-through chambers (see Marron et al. 2009 for details). The diode laser produces linear wavelength scans centred on the selected absorption lines (2291.680 cm^{-1} for $^{13}CO_2$ and 2291.542 cm^{-1} for $^{12}CO_2$).

$[^{12}CO_2]$ and $[^{13}CO_2]$ of available working standards (Air Product, SAS, Paris, France, 0.5% certified for CO_2 concentrations) were 397.22/4.28, 496.94/5.36, 695.80/7.50 and 1189.17/12.83 μmol mol^{-1}, respectively. They were computed from their isotopic composition measured by an isotope ratio mass spectrometer (IRMS, Delta S, ThermoFinnigan, Bremen, Germany).

A first manifold was used to switch between each of three working standards and the chamber inlet and outlet lines. A mean concentration was measured over 20 s for each working standard and for both the reference and sample air streams. A 30-s purge was used in each case. Five minutes were necessary for the complete measurement of the sequence (three working standards followed by reference – sample – reference) on one chamber. A second manifold was used to switch between the seven chamber inlets and outlets, allowing each chamber to be measured every 35 min.

The soil chambers were made of stainless steel and allowed the enclosure of 314 cm^2 of soil (Marron et al. 2009). The chambers were composed of a 12.5-cm high collar, 2.5 cm of which was pushed into the soil, covered with mobile lids allowing the sequential measurements of several collars without removing them from soil. Collars were located 60 cm from the trunks. The trunk chambers were composed of two polymethyl methacrylate half-boxes with semicircular openings to accommodate the trunk (Damesin et al. 2002). The chambers were placed around trunk portions of 20 cm in length and were covered with an insulated aluminium sheet to avoid light and increase in temperature. The two half-boxes were screwed into place and sealed with putty. The inlet of the chamber was made of a 15 cm long PVC tube (internal diameter 41 mm). The surface area of the enclosed trunk segments was computed from the diameter of each extremity, assuming that the trunks were cone shaped. Two chambers were set up at the base of the crown (2.5 m) and at the base of the trunk (0.25 m) of LT1, one at 1.6 m height on the trunk of LT2 and one at 1.3 m height on the trunk of CT. The chamber at the base of the LT1

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trunk was removed after 1 week, whereas the others remained for 2 months.

Air was drawn continuously through both the inlet and the outlet of all chambers using a pump (Membrane pump, GAST DAA-V505-GD). The air flow rate was measured and controlled using mass flowmeters (MC-2SLPM-D, Alicat Scientific) and needle valves, and ranged between 0.25 and 1.25 $dm^3\ min^{-1}$ depending on the rate of respiration, to obtain a concentration difference between inlet and outlet > 50 μmol mol^{-1} for $^{12}CO_2$ and 0.5 μmol mol^{-1} for $^{13}CO_2$, and to remain within the range of $[^{12}CO_2]$ and $[^{13}CO_2]$ of our calibration tanks.

Total CO_2 concentration ($[CO_2]$, μmol mol^{-1}) was calculated from the concentrations of individual isotopologues by

$$[CO_2] = \frac{[^{12}CO_2] + [^{13}CO_2]}{(1 - f_{other})}, \quad (2)$$

where f_{other} is the fraction of CO_2 containing all isotopologues other than $^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$, and is assumed to be 0.00474 (Griffis et al. 2004).

Soil or trunk CO_2 effluxes (F_S and F_T, μmol $m^{-2}\ s^{-1}$) were calculated using either each isotopologue concentration or total CO_2 concentration

$$F_{S,T} = \frac{([CO_2]_{out} - [CO_2]_{in}) \times P \times F}{8.314 \times T \times S_C}, \quad (3)$$

where P is the atmospheric pressure (Pa), F is the flow ($m^3\ s^{-1}$), S_C is the surface of soil or of trunk inside the chamber (m^2), T is the temperature (°K) and 8.314 J $mol^{-1}\ K^{-1}$ is the ideal gas constant.

The isotopic composition of soil or trunk CO_2 effluxes ($\delta^{13}C_{FS}$ and $\delta^{13}C_{FT}$, ‰) was calculated as

$$\delta^{13}C_{FS,FT} = \frac{\frac{[^{13}CO_2]_{out} - [^{13}CO_2]_{in}}{[^{12}CO_2]_{out} - [^{12}CO_2]_{in}}}{R_{PDB}} - 1, \quad (4)$$

where R_{PDB} is the isotopic ratio of Pee Dee Belemnite (0.0111798825).

Label recovered in CO_2 effluxes

The cumulative label recovered in a given efflux (CLR_{FT} and CLR_{FS}), i.e., cumulative label respired by trunk or by root and rhizosphere (root-associated microbes and microbes feeding on root exudates), was calculated by summing the daily average $^{13}CO_2$ effluxes, corrected for the background isotopic composition of effluxes measured on the control tree, and multiplied by the surface (S) of the trunk or the surface of soil beneath which the roots of the trees are

$$CLR(d) = \sum_0^t (F_D \times (A_L - A_C)) \times S, \quad (5)$$

where $CLR(d)$ is the cumulative label recovered at day d after the labelling, F_D is the daily average CO_2 efflux (in $g_C\ m^{-2}\ d^{-1}$) and A_L and A_C are the relative abundance of ^{13}C in the efflux in LT and CT trees. The surface of the soil was delimited by the trenches and the surface of the trunk was estimated from the tree diameter according to allometric equations for beech trees in Hesse (Damesin et al. 2002).

The relative abundance of ^{13}C in any efflux or compartment (A) can be calculated as

$$A = \frac{^{13}C}{^{12}C + {}^{13}C} = \frac{\left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{PDB}}{\left[\left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{PDB}\right] + 1}. \quad (6)$$

The CLR in an efflux can be fitted with a sum of two exponential functions. We chose a sum of two exponential functions rather than a single one because of the shape of the CLR kinetics. This implicitly suggests that the two components were contributing to the efflux. In addition, we added a lag between the pulse labelling and the beginning of its recovery in a given component. We therefore fitted a set of three equations

$$\begin{cases} CLR_{FS,FT}(t) = 0 \quad \text{if} \quad d < L_1 \\ CLR_{FS,FT}(t) = C_1 \times [1 - \exp(-K_1 \\ \quad \times (t - L_1))] \quad \text{if} \quad L_1 < t < L_2 \\ CLR_{FS,FT}(t) = C_1 \times [1 - \exp(-K_1 \times (t - L_1))] \\ \quad + C_2 \times [1 - \exp(-K_2 \times (t - L_2))] \quad \text{if} \quad t > L_2 \end{cases}, \quad (7)$$

where t is time after labelling (in days), K_i are decay constants, C_i are asymptotes, i.e., the total amount of labelled carbon that could be recovered in a given component i, and L_i are lags between labelling and the beginning of the recovery of the label in this component. Equations were fitted using non-linear least-squares regression (PROC NLIN of SAS software, Marquardt–Levenberg method, SAS Institute Inc., Cary, NC). Half-life and mean residence time were ln(2)/K and 1/K, respectively and were expressed in days. We compared the performance of models using a single exponential function or a sum of two exponential functions using an F test (Brown and Rothery 1994, Epron et al. 2004).

Isotopic composition of bulk leaf organic matter

Two twigs, each carrying about 10 leaves, were collected before and just after the pulse labelling. The surface area of the leaves was determined before they were frozen in liquid nitrogen and transported in a Dewar container to the nearby laboratory and then freeze-dried for at least 48 h. The leaves were then weighed and ground in fine powder using a laboratory mill (CB 2200, Cep Industrie - Département SODEMI, St Ouen l'Aumône, France). Total C content and $\delta^{13}C$ of the bulk leaf organic matter were determined using mass spectrometry as above and expressed in ‰. The relative abundance of ^{13}C of leaves was calculated as above (Eq. (6)). SLA was calculated as the ratio of leaf area over leaf dry mass.

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Results

Climate and trunk and soil CO_2 effluxes

Air temperature declined during the labelling and the chase period from summer values at the beginning of September (up to 25 °C during daytime) to winter values at the end of the chase period (< 0 °C during nighttime; Figure 2). During the same time, soil temperature decreased from 16 to 5 °C. A pronounced drop in air temperature was observed between the two labelling dates. The daily sum of PPFD also declined from 1.2 at the beginning of September to values < 0.1 mol m^{-2} d^{-1} in November. Despite some rainfall (84 mm), average soil water content was almost constant in September (19%, data not shown), and was not recorded later.

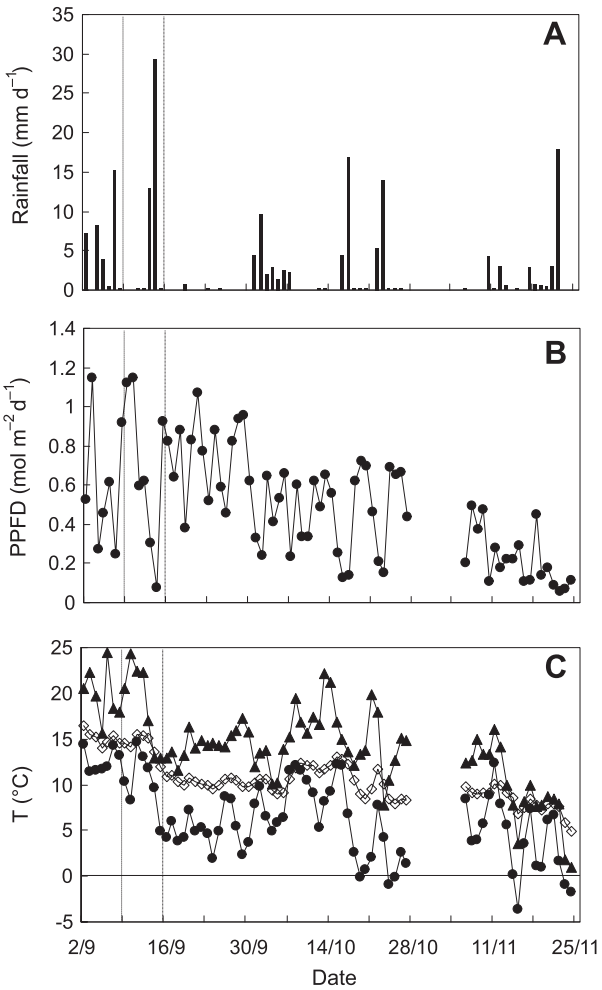

Figure 2. Climatic conditions during the period of 1 September to 30 November 2008. Daily sum of rainfall (A), daily sum of PPFD (B) and daily minimum air temperature (C, closed circles), daily maximum air temperature (C, closed triangles) and daily average soil temperature at 10 cm depth (C, open diamonds). The dotted and the dashed lines indicate the labelling dates of LT1 and LT2, respectively.

Both F_T and F_S (daily averages) exhibited a decline from September to November that followed the decline in temperature (Figure 3). The decline in F_T was more pronounced compared to the declines in F_S as expected from the different decline in air and soil temperatures.

Labelling

Mean air temperature, air humidity and PPFD within the chamber during the labelling period were 21.3 °C (range 12.0–24.4), 72% (50–92) and 974 (360–1960) μmol m^{-2} s^{-1}, respectively, for LT1. The weather was cooler and less sunny for LT2 with mean values of 14.5 °C (6.2–16.9), 90% (60–99) and 502 (135–1470) μmol m^{-2} s^{-1}, respectively. The PPFD measured inside the chamber above the tree was 75% of that measured at the top of the eddy covariance flux tower.

The $^{12}CO_2$ concentration decreased rapidly between the closure of the chamber and the beginning of the injection, at a rate of 0.050 μmol mol^{-1} s^{-1} or 1.48 g_C h^{-1} for LT2 (Figure 4B). The $^{13}CO_2$ concentration decreased at an average rate of 0.113 μmol mol^{-1} s^{-1} (2.34 g_C h^{-1}; Figure 4A) for LT1 and of 0.063 μmol mol^{-1} s^{-1} (1.86 g_C h^{-1}; Figure 4B) for LT2 after the gas cylinder was emptied.

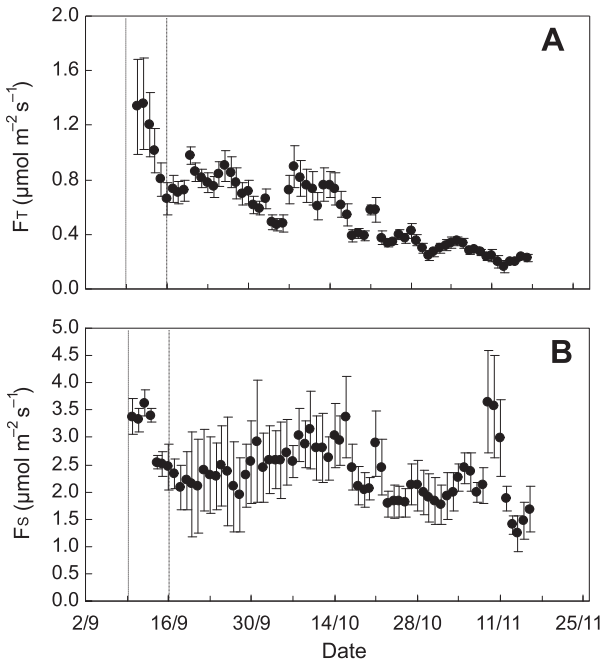

Figure 3. Daily average CO_2 efflux from the trunk (A, F_T) and from the soil (B, F_S). Values are mean of three chambers for trunks (one on each labelled tree and one on the control tree) and of four of six collars for soil (two on each labelled tree and two on the control tree but only four collars were recorded simultaneously. The four soil respiration chambers were moved every week with one chamber on each tree and with one chamber moving between the three trees). Vertical bars are standard deviations. The dotted and the dashed lines indicate the labelling dates of LT1 and LT2, respectively.

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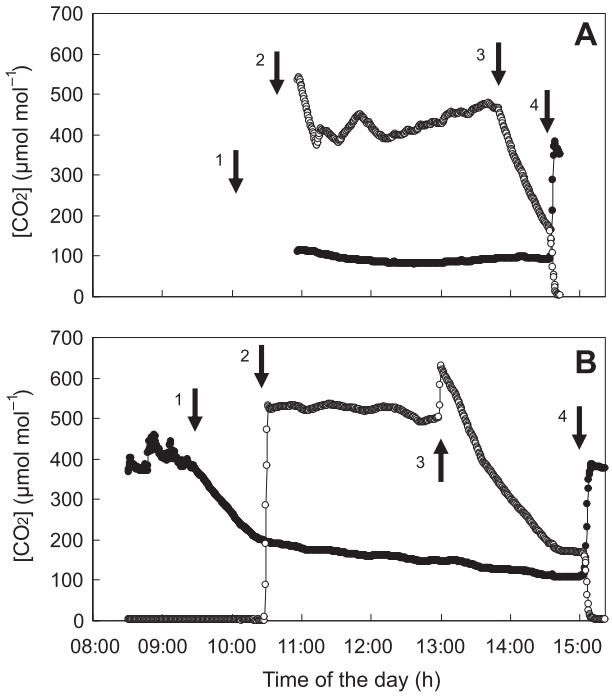

Figure 4. Evolution of $^{12}CO_2$ (closed circles) and $^{13}CO_2$ (open circles) concentrations within the crown labelling chamber (A) during the labelling of the first tree on 9 September and (B) during the labelling of the second tree on 16 September. Vertical arrows indicate from left to right (1) the closing of the chamber, (2) the start of the labelling, (3) the exhaustion of the $^{13}CO_2$ cylinder and (4) the opening of the chamber. We failed to record the data during the first hour of the first labelling experiment.

The total amount of $^{13}CO_2$ taken up by the crown (U, Eq. (1)), assuming negligible leaks, was calculated for LT2 only because initial data were lacking for LT1. Cumulative U was 21.7 L (i.e., 11.96 g of ^{13}C). U could not be estimated precisely for LT1 because of the loss of data.

The residual amount of $^{13}CO_2$ in the chamber at the end of the labelling can be calculated from chamber volume and [$^{13}CO_2$] recorded just before opening the chamber. The amount of injected $^{13}CO_2$ is the difference between the total cylinder volume and this residual amount. It gives 22.6 L (12.45 g of ^{13}C) for LT1 and 21.9 L (12.05 g of ^{13}C) for LT2, respectively.

The isotopic composition of leaves collected just after the end of labelling (30 min) was 1872 ± 233‰ for LT1 and 1075 ± 246‰ for LT2. The foliar biomass of each crown was estimated at 0.90 and 1.98 kg of dry matter for LT1 and LT2, and the carbon content of leaf dry matter was 0.48 ± 0.006 $g_C\ g_{DM}^{-1}$. This represents a total amount of ^{13}C in the leaves of labelled crown of 13.5 and 21.6 g_C for LT1 and LT2. The background of ^{13}C in leaves was obtained knowing the isotopic composition of leaves before labelling and of the control tree (−29.0 ± 0.16‰). The differences between total amount and background were 8.8 and 11.4 g_C for LT1 and LT2. This is equivalent to 16.4 and 20.7 L of $^{13}CO_2$.

$\delta^{13}C$ of trunk CO_2 efflux

Before labelling, the isotopic composition of the trunk CO_2 efflux ($\delta^{13}C_{FT}$) of LT1 and CT was almost identical (−30.5‰ below the crown and −30.4‰ at the base of the trunk for LT1, and −30.1‰ at 1.3 m height for CT). A 10‰-enrichment of $\delta^{13}C_{FT}$ compared to that of the control appeared 11 h after the beginning of the pulse labelling at the base of the crown of LT1 and 13 h at the base of the trunk (Figure 5A). The maximum $\delta^{13}C_{FT}$ was observed at the base of the crown after 39 h (> 4600‰) and at the base of the trunk after 41 h (> 3500‰). This delay between the base of the crown and the base of the trunk corresponds to a phloem sap velocity of about 1 m h^{-1}. $\delta^{13}C_{FT}$ of LT1 declined exponentially during the first two weeks to values around 700‰ and then linearly and slowly during the next weeks to values around 250‰ in mid-November (Figure 5C).

$\delta^{13}C_{FT}$ enrichment of 10‰ for the LT2 (1.6 m height) compared to that of the control tree occurred after 22 h (Figure 5B). The maximum $\delta^{13}C_{FT}$ for LT2 was also delayed compared to LT1 and was observed after 70 h. The maximal $\delta^{13}C_{FT}$ for LT2 (1000‰) was lower than that for LT1, and decreased to < 100‰ after 2 months (Figure 5D). This might reflect the differences in tree size, the pulse label being more diluted in LT2 than in LT1. $\delta^{13}C_{FT}$ of both labelled trees exhibited large diurnal variations, with maximum values at night and minimal values during daytime (Figure 5A and B). These diurnal fluctuations were dampened after several days but were still visible at least 1 month after labelling.

$\delta^{13}C$ of soil CO_2 efflux

Before labelling, the isotopic composition of soil CO_2 efflux ($\delta^{13}C_{FS}$) on LT1 was −26.3‰ and −27.3‰, respectively, for the two collars, in the same range as the values measured on CT (−28.4 and −25.2‰). A 10‰-enrichment in $\delta^{13}C_{FS}$ of LT1 compared to that of control was observed 22 h after the beginning of the labelling (Figure 6A), i.e., about 9 h after the base of the trunk. The maximum enrichment (> 1000‰) was observed 64 h after the beginning of the labelling period, 1 day later than at the base of the trunk.

Recovery of the label in $\delta^{13}C_{FS}$ of LT2 was delayed when compared with that of LT1. $\delta^{13}C_{FS}$ of LT2 was 10‰ above the control 30–35 h after labelling depending on the collars (Figure 6B). The maximum enrichment also differed between collars. It occurred after 72 h (almost at the same time as in the trunk) for the collar exhibiting the highest enrichment (> 1400‰) and after 84 h for the collar exhibiting the lowest enrichment (< 700‰). This might reflect the differences in root contribution to soil CO_2 efflux between the two collars. $\delta^{13}C_{FS}$ initially declined sharply, then slowly during the two chase period months, reaching 20–60‰ (Figure 6C–D).

Irrelevant (i.e., positive) nocturnal values of $\delta^{13}C$ were recorded for both soil and trunk CO_2 effluxes on CT the

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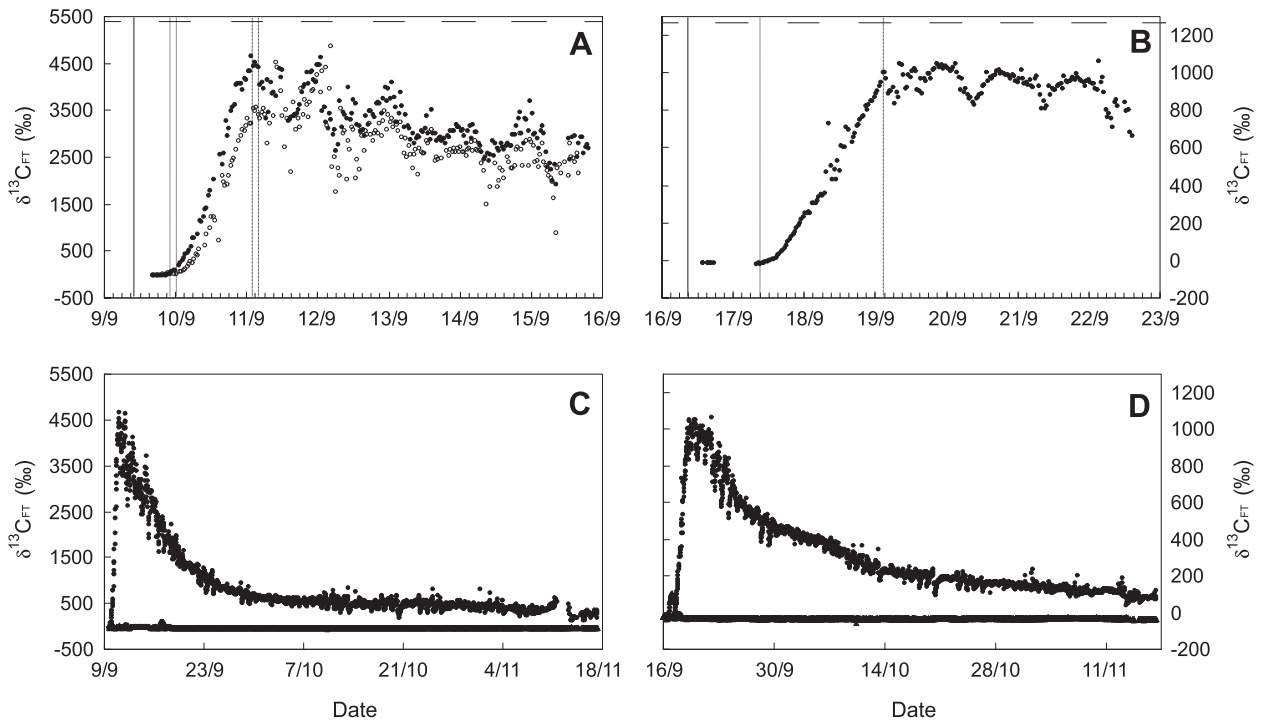

Figure 5. Time courses of the isotope composition of trunk CO_2 efflux ($\delta^{13}C_{FT}$, ‰) after labelling. Comparison of $\delta^{13}C_{FT}$ at the base of the crown (closed symbol) and at the base of the trunk (open symbol) for LT1 during the first week (A), first week of the chase period for LT2 (B) and the 2-month chase period for LT1 (C) and LT2 (D). The top horizontal bars on the upper panels indicate diurnal and nocturnal periods. The vertical lines on the upper panels indicate the beginning of labelling (solid), the beginning of ^{13}C recovery (dotted) and the maximum values of $\delta^{13}C_{FT}$ (dashed), respectively. The base line on the lower panels is the $\delta^{13}C_{FT}$ of CT. Note that the *Y* scale is not the same for LT1 (left) and LT2 (right).

night following the labelling (Figures 5 and 6). These values were surely due to nocturnal accumulation of $^{13}CO_2$ below the canopy because of very stable atmospheric conditions (no wind). Similar, but less pronounced, positive values were also observed the following night.

Label recovery in CO_2 efflux

The rate of increase of the cumulative amount of label recovered in trunk and soil CO_2 effluxes was well predicted using Eq. (7) (Figure 7). A sum of two exponential functions was used, which might suggest that two different pools of substrates with different half-lives are respired by the trunk (Table 1). The sum of two exponential functions predicted CLR in trunk respiration better than that of a single exponential one (Fobs = 1721 for LT1 and 92 for LT2, $P < 0.05$). The half-lives of the fast components were of 2.1 and 8.1 days for LT1 and LT2, while the half-lives of the slow components were longer, 15.2 and 16.2 days for LT1 and LT2. The first component appeared with a lag shorter than a day, while the second component was observed after 6 or 18 days. The total amounts of labelled carbon recovered in trunk CO_2 efflux were 1.17 and 0.51 g_C for LT1 and LT2 (sum of C_1 and C_2 in Table 1).

The cumulative amount of label recovered in soil CO_2 effluxes clearly showed a break point for both trees, and the sum of two exponential functions predicted CLR in soil respiration better than that of single exponential models (Fobs = 2134 for LT1 and 37 for LT2, $P < 0.05$). This effect could once more indicate that two different components were contributing to the label. A lag of more or less 1 day was observed for the first component, while the second component was delayed by 13 (LT2) to 21 (LT1) days. A third component was evident 60 days after labelling on LT1, but the number of data collected after that date was too small for satisfactory fitting. The half-lives of the initial and delayed components of soil CO_2 efflux were 3.3 and 18.2 days for LT1 and 5.2 and 16.7 days for LT2, respectively. The total amounts of labelled carbon recovered in soil CO_2 efflux were 1.30 for LT1 and 1.17 g_C for LT2. The total amounts of carbon recovered in both trunk and soil CO_2 effluxes accounted for 28% and 15% of the ^{13}C retrieved in the foliage just after labelling.

Discussion

Labelling

The crown labelling chamber appeared to be a useful alternative to soil-covering chambers that were used in previous pulse-labelling experiments in the field (Carbone et al. 2007,

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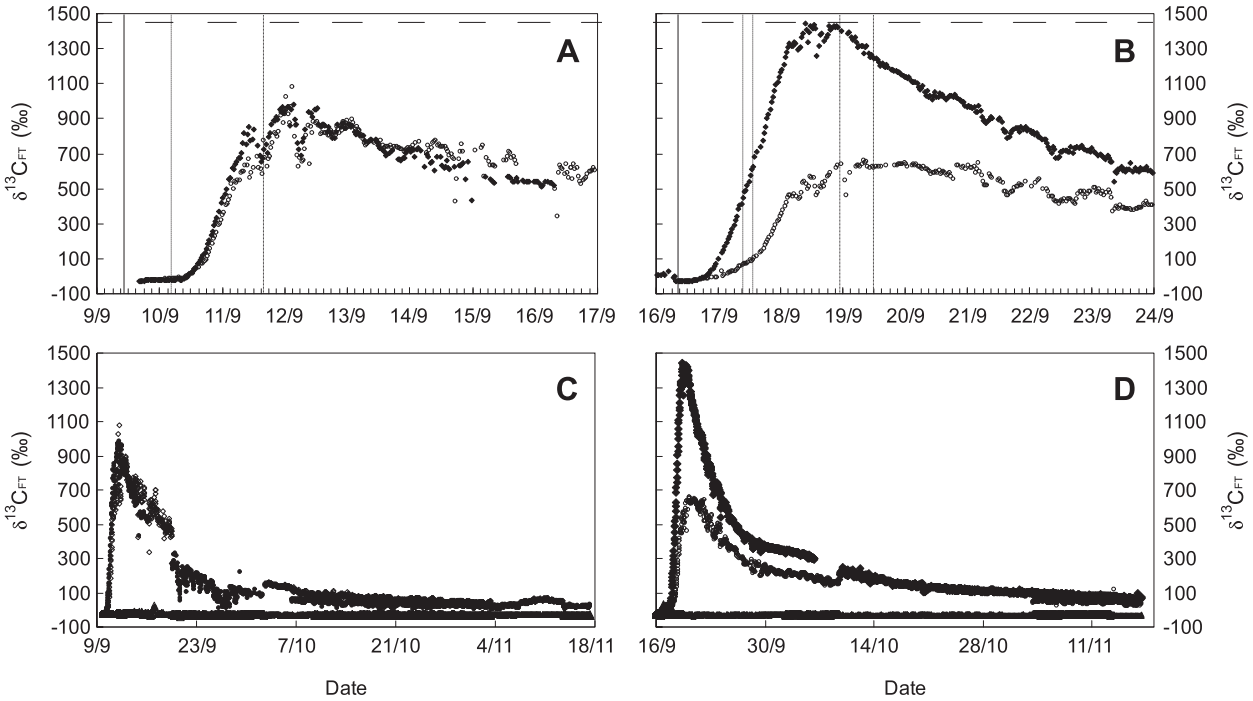

Figure 6. Time courses of the isotopic composition of the soil CO_2 efflux ($\delta^{13}C_{FS}$, ‰) after labelling. First week of the chase period for two collars (open and closed symbols) on LT1 (A) and LT2 (B) and the 2-month chase period for two collars (open and closed symbols) on LT1 (C) and LT2 (D). The top horizontal bars on the upper panels indicate diurnal and nocturnal periods. The vertical lines on the upper panels indicate the beginning of labelling (solid), the beginning of 13C recovery (dotted) and the maximum values of $\delta^{13}C_{FT}$ (dashed), respectively. The base line on the lower panels is the $\delta^{13}C_{FT}$ of CT.

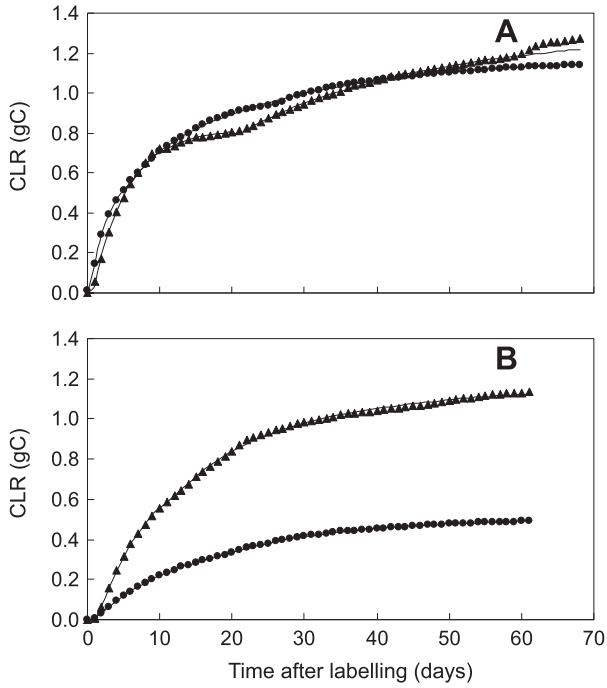

Figure 7. The CLR in the trunk CO_2 efflux (diamonds) and in the soil CO_2 efflux (triangles) for LT1 (A) and LT2 (B). The lines show the fit of Eq. (7) to the experimental data. Adjusted model parameters and the coefficient of determination between observed and simulated values (R^2) are presented in Table 1.

Högberg et al. 2007, Bahn et al. 2009). Firstly, there was no contamination of the soil atmosphere by diffusion of $^{13}CO_2$ into the soil pores that would later have back-diffused into the atmosphere leading to an artefact in soil CO_2 efflux. Secondly, only the target tree was labelled, understorey vegetation remained unlabelled. This is important for studying patterns of below-ground allocation because tree roots, especially root respiration, cannot be easily separated from those of the understorey vegetation. Thirdly, the fact that the chamber only includes the crown enables the labelling of tall trees while maintaining a reasonable chamber volume, and therefore requiring a reasonable amount of $^{13}CO_2$. In addition, as long as the width of the crown increases less than the height of the tree, this type of chamber can be used on tall trees, the limitations being the height of the scaffolds and the access to electricity for the cooling device in remote areas. As far as we know, this is the first in situ pulse-labelling experiment of trees exceeding 8 m in height. Previous in situ labelling experiments were conducted on much smaller trees (2–4 m, Horwath et al. 1994, Carbone et al. 2007, Högberg et al. 2007).

The amount of $^{13}CO_2$ taken up by the crown was calculated by three independent methods: (i) from the change in [$^{13}CO_2$] with time, (ii) the difference between the cylinder volume and the residual amount of $^{13}CO_2$ in the chamber at the end of labelling and (iii) the isotopic composition

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Table 1. Parameters and results of the fit of Eq. (7) (CLR as a function of time after labelling) in trunk and soil CO_2 effluxes. Adjusted parameters ± 95% confidence interval and the coefficient of determination between observed and simulated values (R^2) are given. K are decay constants, C are asymptotes, i.e., the total amount of labelled carbon that was recovered in a given component and L are lags between labelling and the beginning of the recovery of the label for each component.

		First component			Second component			
		C (g_C)	L (d)	K (d^{-1})	C (g_C)	L (d)	K (d^{-1})	R^2
LT1	Trunk	0.66 ± 0.01	0.23 ± 0.04	0.330 ± 0.015	0.51 ± 0.01	6.46 ± 0.28	0.046 ± 0.001	0.999
	Soil	0.82 ± 0.01	0.76 ± 0.03	0.213 ± 0.003	0.48 ± 0.01	21.49 ± 0.23	0.038 ± 0.002	0.999
LT2	Trunk	0.41 ± 0.00	0.95 ± 0.03	0.085 ± 0.002	0.10 ± 0.01	18.00 ± 0.30	0.043 ± 0.002	1.000
	Soil	0.81 ± 0.03	1.38 ± 0.09	0.134 ± 0.011	0.36 ± 0.03	12.66 ± 0.51	0.041 ± 0.004	0.999

of leaves just after labelling. The first two estimations were very similar but both could have been affected by leaks, whereas method (iii) gave slightly lower values. This was expected because sugar export, leaf respiration and $^{12}CO_2$ uptake were likely to dilute the ^{13}C content of leaves. According to these estimates, the labelling device was very efficient since more than 70–90% of the injected $^{13}CO_2$ (corrected for the amount of $^{13}CO_2$ remaining in the chamber) was recovered in the bulk leaves 30 min after the end of labelling.

High-frequency tracking of ^{13}C in respiratory effluxes during the chase period

The TDLAS has proved its reliability for the measurement of the natural abundance of ^{13}C in the atmosphere in the field. Its high temporal resolution allows in situ determination of isotopic CO_2 mixing ratios, the isotopic signature of nocturnal ecosystem respiration and the net ecosystem exchange of $^{12}CO_2$ and $^{13}CO_2$ (Bowling et al. 2003, Griffis et al. 2004, 2005). It has also given more insight into the temporal variations of the isotopic composition of soil respiration, suggesting changes in the relative contribution of respiratory sources (Marron et al. 2009). TDLAS measures $^{12}CO_2$ and $^{13}CO_2$ concentrations separately, rather than isotopic ratios. Values of $\delta^{13}C$ of respired CO_2 as high as $+5000‰$ can be measured in flow-through chambers as long as the $^{13}CO_2$ concentration in the outlet of the chamber remains within the [$^{13}CO_2$] range of the calibration gas. As TDLAS measures specifically $^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$, it does not take into account all the CO_2 isotopologues, such as $^{12}C^{18}O^{16}O$ and $^{12}C^{17}O^{16}O$, for example, (Griffis et al. 2004). The calculation of total CO_2 concentration required a correction (see Eqn. (2)), by assuming the contribution of all other isotopologues to be 0.00474 (f_{other}). This value comes from isotopic abundances used in the high-resolution transmission molecular absorption database (HITRAN, http://www.cfa.harvard.edu/hitran//) and it is related to natural abundance. One might expect that fractionation occurring during photosynthesis, carbohydrate metabolism, transport and respiration would affect f_{other} in the respiratory efflux. More importantly, f_{other} of the labelling gas (0.0116) and of the atmosphere are different. As the respiratory substrates are a mixture of molecules produced before, during and after the pulse labelling, and as CO_2 at the outlet of the chambers is a mixture of atmospheric CO_2 and respired CO_2, f_{other} at the chamber outlets is almost unpredictable. However, using 0.0116 for respired CO_2 and 0.00474 at the inlet for calculating [CO_2] at the outlet of the chamber only slightly changed the absolute values of CO_2 efflux (< 1%).

The TDLAS approach allows the capture of temporal variability at an infra-daytime scale (Bowling et al. 2003, Bahn et al. 2009, Marron et al. 2009). The large diurnal variation we observed in $\delta^{13}C$ of both the soil and trunk CO_2 effluxes was also observed in the soil respiration of alpine grassland (Bahn et al. 2009). It was consistent with diurnal fluctuations of $\delta^{13}C$ (0.5–1.5‰, natural abundance) of the soil CO_2 efflux in a previous study on this site, which also showed maximal values in the middle of the night and minimal values in the early afternoon (Marron et al. 2009). The diurnal variations we observed might reflect a remobilization of stored carbohydrates to sustain respiration during the night and the use of new unlabelled assimilate derived from ongoing assimilation during daytime (Gessler et al. 2008, Bahn et al. 2009). It is also possible that the diurnal variations in $\delta^{13}C$ reflect diurnal changes of the contribution of different sources of CO_2. Day-time F_T might be a mixture of high-labelled CO_2 respired locally and less-labelled CO_2 transported by the xylem sap from the root or the soil, while night-time local respiration would contribute to night-time F_T (Teskey and McGuire 2005). Similarly, diurnal shifts in the relative contributions of both autotrophic (more labelled) and heterotrophic (less labelled) components of F_S might account for the diurnal variations in $\delta^{13}C_{FS}$ (Kodama et al. 2008), but higher relative contributions of root respiration are expected during daytime than during nighttime (Marron et al. 2009).

Kinetics of label recovery and carbon allocation

The high measurement frequency of $^{12}CO_2$ and $^{13}CO_2$ (one measurement every 35 min) enables us to describe the kinetics of label recovery in trunk and soil respiration. The time lag for detecting $^{13}CO_2$ in F_T and F_S is thought to reflect the time required to transfer carbohydrates from

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the crown to the trunk tissue or to the roots. This time lag was higher for LT2 than for LT1, supporting the hypothesis of a reduced velocity of photosynthate transport due to much lower air temperature at the time of LT2 labelling than at the time of LT1 labelling. In LT1, the 2-h delay observed between the chamber at the base of the crown and the chamber at the base of the tree (2.25 m distance) is consistent with a phloem sap velocity of about 1 m h^{-1} (Peuke et al. 2001, Keitel et al. 2003). Temperature affected the velocity of photosynthate transport between the crown and the trunk (0.2 and 0.95 days for LT1 and LT2, respectively) much more than in the root system, as the difference in time lag between F_T and F_S was almost the same for the two labelled trees (about half a day). This was expected because the drop in maximal air temperature between the two labelling experiments (10 °C) was much more important than the change in soil temperature (3 °C). The decrease in phloem sap velocity could be explained by a direct effect of temperature on phloem sap mobility and by an indirect effect through a decrease in photosynthesis and phloem loading. Time lags of 2–4 days were reported for F_S in 3–4 m tall black spruce (Carbone et al. 2007), poplar (Horwath et al. 1994) and Scots pine (Högberg et al. 2007) trees. The discrepancies between previous estimates and our results could be attributed to difference between gymnosperm and angiosperm trees or to a better time resolution in the TDLAS-based measurements. A time lag of 0.8 day and a phloem sap velocity of about 1 m h^{-1} for LT1 (see above) suggest a path length of about 20 m that is twice the size of the trees. These findings corroborate previous results showing a close coupling between photosynthesis and soil CO_2 efflux (Ekblad and Högberg 2001, Bowling et al. 2002, Knohl et al. 2005).

The fact that the CLR in an efflux was better described with a sum of two exponential functions than with a single one, implicitly suggests that two components were contributing to both trunk and soil effluxes. The first components had a shorter mean residence time for the first labelled tree than for the second one (MRT 2–3 days and 5–8 days, respectively), and might reflect a direct use of labelled photosynthate in the respiratory metabolism that is rapidly diluted by recent photosynthate. The difference in MRT between the two labelled trees might reflect a slowdown of photosynthesis (i.e., a lower dilution) when temperature dropped just before the second labelling. Transitory storage and rapid remobilization cannot be excluded (see above discussion on the infra-day variations). The second component that contributed to the effluxes after a lag time of 6–21 days and had a MRT of 22–26 days might correspond to the use of stored carbohydrate later in the season when the supply of current photosynthesis no longer supported trunk or root respiration. Similar patterns were observed for F_T and F_S suggesting that the second component of F_S could also be related to the root compartment. However, we cannot fully exclude that the second component of F_S could be associated to the heterotrophic component of soil respiration and to the onset of decomposition of labelled organic substrate (turnover of mycorrhiza hyphae or fine roots, turnover of microbes fed on labelled root exudates).

The amounts of label recovered in soil CO_2 efflux and in trunk CO_2 efflux were different between the two trees (15% of the assimilated $^{13}CO_2$ in soil respiration for LT1 and 10% for LT2, and 13% of the assimilated $^{13}CO_2$ in trunk respiration for LT1 and only 5% for LT2). Therefore, the fraction of the label that was allocated to both trunk and soil respiration represented 28% of the assimilated $^{13}CO_2$ for LT1 and 15% for LT2. Allocation to crown (branches and leaves) respiration was not estimated in this study. On an annual basis, branch and leaf respiration of beech accounted for 43% of autotrophic respiration at this site, and root and stem respiration accounted for 57% (Granier et al. 2000, Damesin et al. 2002). Using these ratios, the fraction of label allocated to whole tree respiration could be estimated at 26–49% of the assimilated $^{13}CO_2$. As labelling occurred late in the growing season (radial growth surveyed by means of micro-dendrometers ended mid-September at this site), substantial allocation to storage was expected (Mordacq et al. 1986, Barbaroux et al. 2003), especially for the second tree that was labelled later in the season after a marked drop in air temperature.

Conclusions

The large crown labelling chamber we described was suitable to deliver a 3-h pulse of $^{13}CO_2$ for the labelling of tall trees in the field, without causing contamination of the soil atmosphere that was observed with soil-covering chambers. The high temporal resolution offered by tuneable diode laser spectrometers enables accurate assessments of the time lag between photosynthetic assimilation by the crown and CO_2 release in the trunk and in the soil, as well as precise determinations of the half-life of labelled carbon in respiratory effluxes. A stimulating question is whether the time constant can be predicted in mechanistic terms.

The TDLAS approach allows the capture of temporal variability at a scale that could not be reached with classical IRMS or AMS approaches. But there is a compromise between the frequency of measurements and the number of chambers that can be measured sequentially. Our chase experiments were not designed to account for the spatial variability of soil CO_2 efflux in the vicinity of the labelled trees. This could lead to high uncertainties in the estimation of the fraction of the label that was allocated to root respiration.

The present pulse-labelling experiment was carried out in September, late in the growing season when growth in both height and diameter has ceased. Allocation patterns to below-ground respiration and trunk respiration may be different during the periods of active growth. The experiments were conducted on only two trees, and are therefore 'case studies' from which general statements on carbon

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allocation should be derived with caution. This study has opened the door for future experiments on several species. Pulse-labelling experiments over the whole growing season are required to capture seasonal changes in carbon allocation patterns.

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In situ assessment of the velocity of carbon transfer by tracing ^{13}C in trunk CO_2 efflux after pulse labelling: variations among tree species and seasons

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Summary

- Phloem is the main pathway for transferring photosynthates belowground. *In situ* ^{13}C pulse labelling of trees 8–10 m tall was conducted in the field on 10 beech (*Fagus sylvatica*) trees, six sessile oak (*Quercus petraea*) trees and 10 maritime pine (*Pinus pinaster*) trees throughout the growing season.
- Respired $^{13}CO_2$ from trunks was tracked at different heights using tunable diode laser absorption spectrometry to determine time lags and the velocity of carbon transfer (*V*). The isotope composition of phloem extracts was measured on several occasions after labelling and used to estimate the rate constant of phloem sap outflux (k_P).
- Pulse labelling together with high-frequency measurement of the isotope composition of trunk CO_2 efflux is a promising tool for studying phloem transport in the field. Seasonal variability in *V* was predicted in pine and oak by bivariate linear regressions with air temperature and soil water content. *V* differed among the three species consistently with known differences in phloem anatomy between broadleaf and coniferous trees.
- *V* increased with tree diameter in oak and beech, reflecting a nonlinear increase in volumetric flow with increasing bark cross-sectional area, which suggests changes in allocation pattern with tree diameter in broadleaf species. Discrepancies between *V* and k_P indicate vertical changes in functional phloem properties.

Introduction

Soil–vegetation–atmosphere transfer models are key tools for predicting carbon exchange responses to climate change between the biosphere and the atmosphere, and for studying feedback between climate and ecosystem functions. However, such models still suffer from a lack of mechanistic integration of carbon allocation (Cannell & Dewar, 1994; Friedlingstein *et al.*, 1999; Magnani *et al.*, 2000; Litton *et al.*, 2007). Allocation of assimilated carbon among organs is affected by the environment and this affects tree growth, the contribution of each organ to autotrophic respiration, carbon transfer to the rhizosphere and, *in fine*, carbon sequestration in the ecosystem (Magnani *et al.*, 2002; Giardina *et al.*, 2003).

The transfer of photosynthetic products belowground represents between 25–63% of gross primary productivity (Litton *et al.*, 2007) and fulfils energy requirements for metabolic processes occurring in roots and in the (myco)rhizosphere. Previously, temporal variations in soil respiration were mostly ascribed to environmental drivers (Lloyd & Taylor,

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1994; Davidson *et al.*, 1998; Epron *et al.*, 1999), but now there is growing evidence that soil respiration is strongly linked to plant activities (Högberg *et al.*, 2001; Högberg & Read, 2006; Marron *et al.*, 2009; Plain *et al.*, 2009; Bahn *et al.*, 2010).

Phloem is the main pathway for transferring photosynthates belowground. Increasing our understanding of carbon allocation between sinks requires better characterization of phloem transport of photosynthates (Minchin & Lacointe, 2005). In trees, the time lag between canopy photosynthesis and soil respiration depends mainly on the path length and on the velocity of phloem transport in the trunk and through the root system. This time lag ranges between 1 and 5 d depending on the height of the tree (Kuzyakov & Gavrichkova, 2010; Mencuccini & Hölttä, 2010). This velocity is thought to differ among species as a result of differences in phloem anatomy and in response to changes in environmental conditions (Kuzyakov & Gavrichkova, 2010), but very few data are currently available (Peuke *et al.*, 2001; Helfter *et al.*, 2007). Drought is known to drastically reduce carbon transport belowground in beech (*Fagus sylvatica*) seedlings (Ruehr *et al.*, 2009) and preliminary observations indicated that a 10°C drop in air temperature may have strongly reduced the velocity of photosynthate transport between the canopy and the base of the trunk in beech trees (Plain *et al.*, 2009). However, very little is known about seasonal variations in, and the impact of environmental factors on, phloem transport in field-grown trees (Van Bel, 2003).

The first attempts to estimate the *in situ* velocity of carbon transfer belowground relied on time series analysis or lag correlation analysis of temporal fluctuations of soil respiration or its ^{13}C composition (Craine *et al.*, 1999; Ekblad *et al.*, 2005; Gaumont-Guay *et al.*, 2008; Marron *et al.*, 2009). However, the close coupling between soil respiration and microclimate may reflect not only a direct transfer of carbon from the canopy to the soil but also a several orders of magnitude faster transfer of information through pressure–concentration waves linked to dynamic changes in phloem turgor (Thompson & Holbrook, 2004; Mencuccini & Hölttä, 2010). In addition, mixing of different CO_2 sources and post-photosynthetic carbon isotope fractionation may overshadow the coupling between canopy photosynthesis and soil respiration (Kodama *et al.*, 2008).

Pulse labelling of plants with isotopically enriched (or depleted) CO_2 is therefore seen as the most appropriate method for studying the velocity of carbon transfer in plants (Dawson *et al.*, 2002; Kuzyakov & Gavrichkova, 2010). Until recently, labelling experiments were restricted to young tree saplings in pots (Horwath *et al.*, 1994; Pumpanen *et al.*, 2009; Ruehr *et al.*, 2009), or to small trees in the field (Mordacq *et al.*, 1986; Carbone *et al.*, 2007; Högberg *et al.*, 2007; but see Plain *et al.*, 2009) and the ability to precisely determine the velocity of carbon transfer was limited by constraints on the frequency of measurement of ^{13}C during the chase period. The recent development of laser-based infrared gas analysers has increased by several orders of magnitude the measurement frequency of $^{13}CO_2$ in respiratory fluxes in pulse labelling experiments (Bahn *et al.*, 2009; Plain *et al.*, 2009).

The aim of this study was to estimate the vertical velocity of carbon transfer inside the trunks of two deciduous broadleaf species (beech and sessile oak (*Quercus petraea*)) and one coniferous evergreen species (maritime pine (*Pinus pinaster*)). We compared time lags between the photosynthetic assimilation of $^{13}CO_2$ and its recovery in trunk CO_2 efflux recorded continuously by tuneable diode laser absorption spectrometers three to four times during the growing season. Differences in time lags in trunk CO_2 efflux recorded at different heights along the trunk were used to estimate the velocity of phloem transport of ^{13}C-labelled compounds in trees 8–10 m tall. We further compared time lags and velocities with the dynamics of the label recovered in the phloem sap. We hypothesized that the velocity of carbon transfer is higher in angiosperm than in gymnosperm species because of differences in phloem anatomy and that velocity changes seasonally according to changes in temperature and soil water content.

Materials and Methods

Study sites

The study was conducted on beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* Matt Liebl.) and maritime pine (*Pinus pinaster* Ait.). The beech stand is 20-yr-old natural regeneration located in the state forest of Hesse (48°40′N, 7°04′E, 300 m elevation) on a luvisol. Mean annual air temperature and precipitation are 9.2°C and 820 mm, respectively. The oak stand is 15-yr-old natural regeneration located in the state forest of Barbeau (48°2′N, 02°47′E, 90 m elevation) on a gleyic luvisol. Mean annual air temperature and annual rainfall are 12.9°C and 680 mm, respectively. The pine plantation was established in 1998 (12 yr old) at the INRA (Institut National de Recherche Agronomique) domain of Pierroton (44°45′N, 0°42′W, 60 m elevation) on a sandy spodosol with an initial tree spacing of 2 × 2 m. The plot was thinned in November 2008 by removing one line out of two. Mean annual air temperature and precipitation are 13.3°C and 930 mm, respectively. The height of dominant trees was 8–10 m in the three plots with average diameters at 1.3 m height (dbh) of 6.7, 8.7 and 13.2 cm for beech, oak and pine, respectively.

Experimental design

Two trees were selected for each labelling date at all sites, giving a total number of 10 beeches, six oaks and 10 pines (Supporting Information Table S1). Labelling was conducted

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in September 2008 and May, July and August 2009 for beech, in May, July and September 2009 for oak and in June, August and November 2009 and February 2010 in pine in order to cover the different growing phases of each species: the beginning of radial growth, active radial growth and the termination of radial growth for the three species, and additionally the rest period in winter for the evergreen pine.

Two additional beeches and pines that were exposed to rainfall exclusion were labelled in August (beech) or in November (pine). Rainfall exclusion roofs, made with polyethylene film and supported by a woody frame (3 m × 3 m), were installed 1.5 m above the forest floor on two beech trees and two pine trees to divert rainfall from the soil delimited by the trench. The roofs were installed in April 2009 for beech, excluding rainfall throughout the growing season, and in September 2010 for pine, excluding rainfall after the summer drought.

A trench 0.5–0.6 m deep was dug in winter around each tree at least 5 months before labelling. This depth corresponds to the presence of a compact clay horizon for both beech and oak, and to an indurate soil layer (alios; hardpan) for pine, which limit vertical root development. We did not assess to what extent trenching affected the root systems of the trees, but we assumed that the root system had recovered from the trenching stress at the time of labelling. The trench was lined with a polyethylene film and filled back. All roots and root exudates within this soil volume therefore originated from the isolated tree, and were contained in this trench volume. The area delimited by the trench depended on the density of the stand; that is, on the spacing between trees. The area averaged 3 m^2 in the beech (2.1–4.0 m^2) and oak (2.2–5.5 m^2) stands, and was 6 m^2 in the pine plantation. These areas were higher than the mean area per tree based on stand density.

Microclimate parameters, including photosynthetic photon flux density (PPFD; measured using Delta T-BF2 (Delta-T devices, Cambridge, UK) for beech; a homemade quantum sensor based on a gallium arsenide photodiode for oak; and DBE (Solems, Palaiseau, France) for pine), air temperature and relative air humidity (T_A and RH, respectively; Vaisala HMP45, Vaisala, Helsinki, Finland), were recorded using data loggers (Campbell Scientific, Logan, UT, USA) at a 10–60 s pace and averaged half-hourly. Soil water content (SWC) in the vicinity of each labelled tree inside the area delimited by the trench was recorded half-hourly at 30 cm depth for pine with time domain reflectometry (TDR) probes (CS616; Campbell Scientific) and at 10 cm depth for oak with an impedance probe (ML2x ThetaProbes; Delta-T Device), and measured once a week at 15 cm depth for beech using TDR probes (Trase; SoilMoisture Equipment Corp., Santa Barbara, CA, USA).

Predawn leaf water potential was measured once on three to four leaves per tree just before labelling at the end of August for beech (four trees) and at the beginning of September for oak (two trees) using a Scholander-type pressure chamber (PMS Instrument, Corvallis, OR, USA).

Pulse labelling

Each pulse labelling was performed using the whole-crown labelling chamber previously described (Plain *et al.*, 2009). The whole crown of the tree was inserted into a 20–25 m^3 chamber made of 200-μm polyane film and held up by two 12-m-high stainless steel scaffolds erected on either side of the tree. The air temperature inside the crown labelling chamber was recorded, controlled and maintained at the outside air temperature. For oak and beech, this was done by circulating air at a rate of 1200 m^3 h^{-1} through an air- and water-cooled condensing axial fan outdoor unit connected to an air conditioner (AXCZ 221 and BSH-BZ 221; HITECSA, Vilanova i la Geltrú, Spain) (see Plain *et al.*, 2009 for details), while for pine, two air conditioners (Bodner and Man Pap 3500 W, Castorama, Templemars, France) and three axial fans were directly inserted into the labelling chamber.

Evolution of both $^{12}CO_2$ and $^{13}CO_2$ concentrations ($[^{12}CO_2]$ and $[^{13}CO_2]$, respectively) inside the labelling chamber was monitored simultaneously with a $^{12}CO_2/^{13}CO_2$ infrared gas analyser (S710; SICK/MAIHAK, Reute, Germany; accuracy 5%) calibrated by diluting pure CO_2 from gas tanks having different $^{13}C/^{12}CO_2$ (10, 50 and 100%) in a stream of CO_2 free air using mass flow controllers. The $^{12}CO_2$ concentration decreased to 60–150 μmol mol^{-1} after the chamber was closed because of CO_2 assimilation by photosynthesis. A total amount of 25 l of pure $^{13}CO_2$ (99.299 atom%; Eurisotop; Cambridge Isotope Laboratory Inc., Andover, MA, USA) was then progressively injected at a flow rate adjusted to maintain the $^{13}CO_2$ concentration in the chamber at between 300 and 400 μmol mol^{-1}, except in November and February for pine, when higher $^{13}CO_2$ concentrations were used to compensate for lower photosynthetic activity (800–1000 μmol mol^{-1}). The duration of labelling ranged between 3 and 6 h for beech, 1 and 3 h for oak and 2 and 5 h for pine, depending on tree size and activity.

^{13}C composition of CO_2 efflux

The isotope composition of CO_2 effluxes ($\delta^{13}C_F$) from the trunk was computed from $[^{12}CO_2]$ and $[^{13}CO_2]$ measured at the inlet and outlet of flow-through chambers (Marron *et al.*, 2009; Plain *et al.*, 2009) by tuneable diode laser absorption spectroscopy with a trace gas analyzer (TGA 100A; Campbell Scientific). Air was pumped continuously through the chamber at a flow rate ranging from 300 to 1200 l min^{-1}. A manifold was used to switch between working standards and the chamber inlet and outlet lines. Three standards were measured before measurements were made at the inlet and outlet of the respiration chamber. The mean concentration was recorded over 20 s after a 30-s stabilization period.

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Certified standards of CO_2 in synthetic air (0.5% precision for CO_2 concentrations, Air Products, Paris, France for beech, Air Liquide, Paris, France for oak and DEUSTE Steininger GmbH, Mühlhausen, Germany for pine) were measured for isotope composition every 2–6 months using an isotope ratio mass spectrometer (IRMS; Delta S; ThermoFinnigan, Bremen, Germany) that had been calibrated against IAEA (International Atomic Energy Agency) reference materials. The isotope composition was used to compute the $[^{12}CO_2]$ and $[^{13}CO_2]$ of each working standard. The ranges of available concentrations were 293.2 to 1281.2 μmol mol^{-1} for $^{12}CO_2$ and 3.17 to 13.75 μmol mol^{-1} for $^{13}CO_2$. The flow rate of air through the respiration chambers was adjusted to maintain the $[^{12}CO_2]$ and $[^{13}CO_2]$ at the outlet within the range of the calibration standards. The precision of the instrument at reproducing calibration tank values was 0.3 μmol mol^{-1} for $^{12}CO_2$ and 0.007 μmol mol^{-1} for $^{13}CO_2$.

The isotopic composition of CO_2 effluxes ($\delta^{13}C_F$; ‰) was calculated as:

$$\delta^{13}C_F = \frac{\frac{[^{13}CO_2]_{out} - [^{13}CO_2]_{in}}{[^{12}CO_2]_{out} - [^{12}CO_2]_{in}}}{R_{VPDB}} - 1, \qquad \text{Eqn 1}$$

(R_{VPDB}, the isotopic ratio of Vienna Pee Dee Belemnite (VPDB; 0.011179602)).

The trunk chambers on beeches and oaks were composed of two polymethylmethacrylate half-boxes with semicircular openings to accommodate the trunk (Damesin *et al.*, 2002). The trunk chambers on pines were composed of polycarbonate sheet wound around the trunk on foam rubber to form a cylinder (height 0.1–0.2 m; distance to bark 0.03 m). The vertical distance between the trunk chambers and the soil surface was measured. One chamber was set up at the base of the trunk (0.2–0.7 m from the soil) and at the base of the crown (2.1–3.3 m for beech and oak, and 3.8–5.3 m for pine). $\delta^{13}C_F$ was measured every 40–80 min for at least 5 d and up to 2 wk.

Carbon isotope composition of phloem extracts

Two small disks of bark (12 mm diameter for beech and pine and 14 mm for oak) were collected on labelled trees at a height of 1.3 m at 9 h Coordinated Universal Time (UTC) using a corer (Gessler *et al.*, 2004). Disk sampling was repeated 4–5 times during the 10-d chase following the $^{13}CO_2$ labelling pulse. Additional samples were collected before the labelling and on a nearby unlabelled tree. The inner bark tissue was placed in a 10-ml vial containing 2 ml of ultrapure water after removal of the outer part of the bark (periderm), and it was left to incubate for 5 h at ambient temperature. Then the bark pieces were separated from the phloem extract solution, dried for several days in an oven at 60°C, and then weighed. The phloem extract solution was filtrated on a nylon cartridge (Whatman, 0.2 μm Nyl.; diameter 25 mm; ref. 17 463 433) and the filtrate was vacuum-evaporated for 4 h (Maxi-Dry plus, Heto-model DW1, 0-110; Heto-Holten A/S, Allerod, Denmark). Dried extracts were weighed and then diluted in ultra-pure water proportionally to their dry mass. An aliquot of 60 μl of the resulting solution (containing *c.* 0.8–1 mg of dried phloem extract) was transferred into tin capsules (Elemental Microanalysis, Cambridge, UK; 6 × 4mm; ref. D1006, BN/139877), freeze-dried (Lyophilizer Christ; Alpha 1-2 LD plus, Osterode am Harz, Germany), weighed and analysed for carbon isotope composition and total carbon using an elemental analyser (NA 1500 NCS; Carlo Erba, Milan, Italy) coupled to a Delta-S isotopic ratio mass spectrometer (Finnigan-Mat; Thermoquest Corp., San Jose, CA, USA) for beech and pine samples and to a VG Optima IRMS (Fison, Villeurbanne, France) for oak samples. The carbon isotope composition of the phloem extract ($\delta^{13}C_P$) was expressed in delta notation (in ‰) relative to the VPDB standard.

The thickness of the inner bark tissues of all labelled trees and a few additional unlabelled trees was assessed on additional phloem disks collected at the same time for all trees at a given site. Measurements were made with a ruler at 0.5 mm accuracy for oak and with a calliper at 0.1 mm accuracy for beech and pine.

Data analysis

The time lag (L_T; h) between the start of the labelling and the appearance of ^{13}C in CO_2 efflux in each chamber was computed by fitting a quadratic function to the relationships between $\delta^{13}C_F$ (‰) and the time after labelling (t; h; Fig. 1a) using nonlinear least-squares regression (PROC NLIN of the SAS software; Marquardt–Levenberg method; SAS Institute Inc., Cary, NC, USA):

$$\begin{cases} \delta^{13}C_F = a \ \text{ if } t < L_T \\ \delta^{13}C_F = a + b\,(t - L_T) + c\,(t - L_T)^2 \ \text{ if } t \geq L_T \end{cases} \qquad \text{Eqn 2}$$

with a (‰) matching the pre-labelling isotope composition of the efflux. Time lags for the chamber at the base of the trunk (bottom chamber) and at the base of the crown (top chamber) were further denoted L_{T1} and L_{T2}, respectively.

The velocity of carbon transfer in the trunk (V; m h^{-1}) was calculated as

$$V = \frac{d}{L_{T1} - L_{T2}}, \qquad \text{Eqn 3}$$

(d (m), the distance between the two chambers.) Our calculation assumes that, if transferred carbon is not at least partly respired immediately or the diffusion time of CO_2 across the bark is not negligible, these delays are similar for the two chambers, so they are cancelled by the subtraction of the two time lags.

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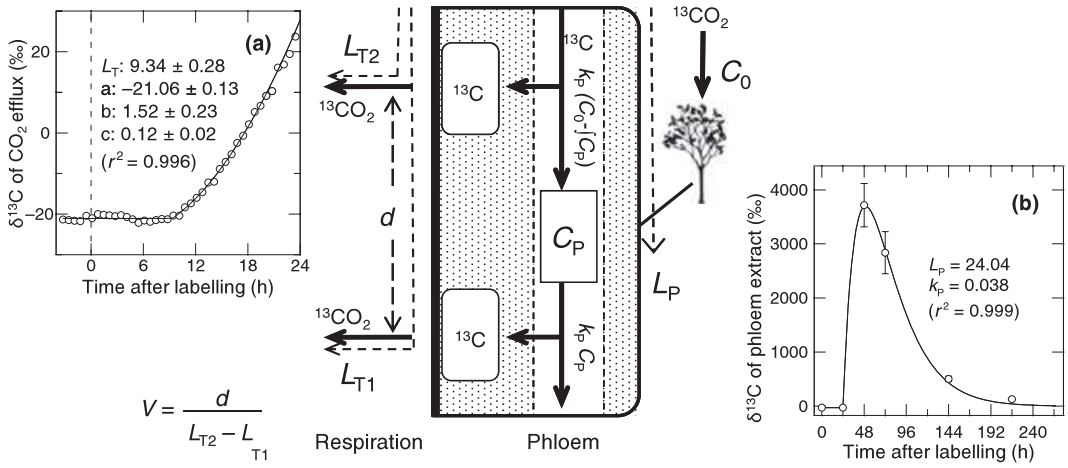

Fig. 1 Schematic diagram showing respiration at two positions along the trunk on the left and the flow of labelled carbon through the phloem on the right. (a) Determination of the time lag (L_T) between the start of the labelling and the first appearance of ^{13}C in CO_2 efflux by fitting a quadratic function to the relationships between $\delta^{13}C_F$ and the time from the beginning of the labelling (Eqn 2). The example shows $\delta^{13}C_F$ in trunk CO_2 efflux (top chamber) in oak in September 2009. (b) Determination of the time lag (L_P) between the start of the labelling and the first appearance of ^{13}C in the phloem extract using a single pool model, and assuming that outflux from phloem extract follows first-order kinetics with a rate constant k_P and that influx is equal to outflux. The example shown is for pine in August 2009.

The kinetic of label recovered in the phloem extract was described using a single pool model that was fitted to the observed $\delta^{13}C_P$ values (Fig. 1b). The label that left the crown arrived after a time lag (L_P) into the phloem extract collected at a height of 1.3 m above the ground. We assumed that the outflux of label from the crown and from phloem extract collected at a height of 1.3 m followed a first-order kinetic with the same rate constant (k_P) and that the influx into the phloem extract was equal to the outflux from the crown. The rate of change of label in the phloem extract with time (t) is given by:

$$\begin{cases} \frac{dC_P(t)}{dt} = 0 \quad \text{if } t<L_P \\ \frac{dC_P(t)}{dt} = k_P\left[C_0 - \int_{L_P}^{t} C_P(t)\right] - k_P\, C_P(t) \quad \text{if } t \geq L_P, \end{cases} \qquad \text{Eqn 4}$$

($C_P(t)$, the amount of ^{13}C label in the phloem extract at time t after labelling; C_0, the total amount of label that has flowed out of the crown through the phloem.)

The analytical solution is:

$$\begin{cases} C_P(t) = C_P(0) \text{ if } t < L_P \\ C_P(t) = C_P(0) + (C_0 - C_P(0)) \cdot k_P \cdot (t - L_P) \cdot \\ \qquad \exp(-k_P \cdot (t - L_P)) \quad \text{if } t \geq L_P \end{cases} \qquad \text{Eqn 5}$$

Because we were only interested in L_P and k_P, we used the difference in $\delta^{13}C_P$ between labelled and unlabelled trees as a proxy for C_P. The differential equation was solved analytically to estimate the values of L_P and k_P by iteratively minimizing the sum of squared differences between measured and predicted $\delta^{13}C_P$ using the Microsoft Excel solver tool. Because of the low number of collected samples, the standard fitting procedure could not be applied satisfactorily so the fit quality was checked visually. We were aware that L_P could not be determined when the peak occurred before the first sampling 24 h after labelling. The mean residence time (MRT) of label in the phloem extract was calculated as $1/k_P$.

Linear regressions were used to analyse the relationships between the different computed parameters (L, L_P, V and k_P). Linear regressions (simple or multiple) were also used to evaluate the relationships between these computed parameters and either tree dimension (dbh) or environmental variables (PPFD, RH, T_A and SWC). The coefficient of determination was tested with a two-tailed test for significance of the regression (the REG procedure of the SAS software).

Analysis of variance was performed to test significant differences in phloem dimension between species and trees nested within species (the GLM procedure of the SAS software).

Results

Environmental conditions

Pulse labelling experiments were performed throughout the growing season for the three species. The daily average temperatures on the labelling dates ranged between 9 and 24°C for beech and pine and between 15 and 24°C for oak. The volumetric SWC ranged between 0.31 and 0.39 m^3 H_2O m^{-3} in the beech stand, except for trees under rainfall exclusion roofs (0.13–0.23 m^3 H_2O m^{-3}). There was no rainfall exclusion treatment in oak, but SWC exhibited a

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more pronounced seasonal variation than in beech, with values ranging from 0.30 m^3 H_2O m^{-3} in May to 0.20 m^3 H_2O m^{-3} in September. In pine (sandy soil), SWC was lower than for oak and beech, ranging between 0.05 and 0.18 m^3 H_2O m^{-3} for control trees and being 0.05 for both trees under rainfall exclusion roofs (Table S1). Predawn leaf water potential values were similar (−0.4 MPa; data not shown) for the four beech trees labelled in August, regardless of whether or not they were subjected to rainfall exclusion, and dropped below −0.9 MPa in oak in early September.

Recovery of ^{13}C in trunk CO_2 efflux

The carbon isotope enrichment of CO_2 efflux ($\delta^{13}C_F$) from the trunk increased after each pulse labelling in all species after a time lag from an average of −25‰ up to 10 000‰. The label, as expected, appeared first in CO_2 efflux at the base of the crown and then at the base of the trunk, and started to decline after several hours or days, depending on the species and the time of year (Fig. 2). The $\delta^{13}C_F$ values measured at peak time were often, but not always, lower for the bottom chamber than for the top chamber (typical examples are shown in Fig. 2). In oak, the difference was visible early in the growing season but not later (Fig. 2; central panels). In beech, there was no clear seasonal pattern for this difference in maximum $\delta^{13}C_F$ between the base of the crown and the base of the trunk. The difference was more pronounced for smaller trees than for bigger trees (Fig. 2; left panels) and was indeed negatively related to dbh (R^2 = 0.58, P = 0.012; data not shown). Rainfall exclusion did not alter the shape of the kinetics of recovery of ^{13}C in trunk CO_2 efflux in beech, while the $\delta^{13}C_F$ values measured at peak time were much lower in pine trees exposed to water exclusion (Fig. 2; Excl. labels). The shape also differed markedly between the growing season and the dormant period for pine (Fig. 2; right panels).

In beech, the time lag in trunk CO_2 efflux (L_{T2}) ranged from 6 to 9 h at the base of the crown (Fig. 3a). The signal appeared *c.* 3 h later at the base of the trunk (L_{T1}; Fig. 3a, Table S1). The time shifts were longer on a few occasions, such as in September 2008, when the mean air temperature after labelling was 9°C (mean temperature ranged between 13 and 24°C for the other labelling experiments on beech). This also occurred in August 2009 on the smallest labelled beech tree (dbh = 4.9 cm). In oak, L_{T2} values were *c.* 5–6 h at the base of the crown and 8–11 h at the base of the trunk (L_{T1}) in May and July. Signal appearance was delayed in September (*c.* 9 and 16 h, respectively; Fig. 3b). In pine, the time lags in trunk CO_2 efflux were more variable (from 4 to 47 h at the base of the crown; Fig. 3c) and the time shift between the top and the bottom chambers was much higher than for the other species, with time lags of 30–80 h at the base of the trunk.

A shift of similar amplitude was observed for the maximum $\delta^{13}C_F$ occurring at the base of the crown and at the base of the trunk, with an averaged delay of 5 h in beech (40 h vs 45 h on average, respectively), 10 h in oak (45 h

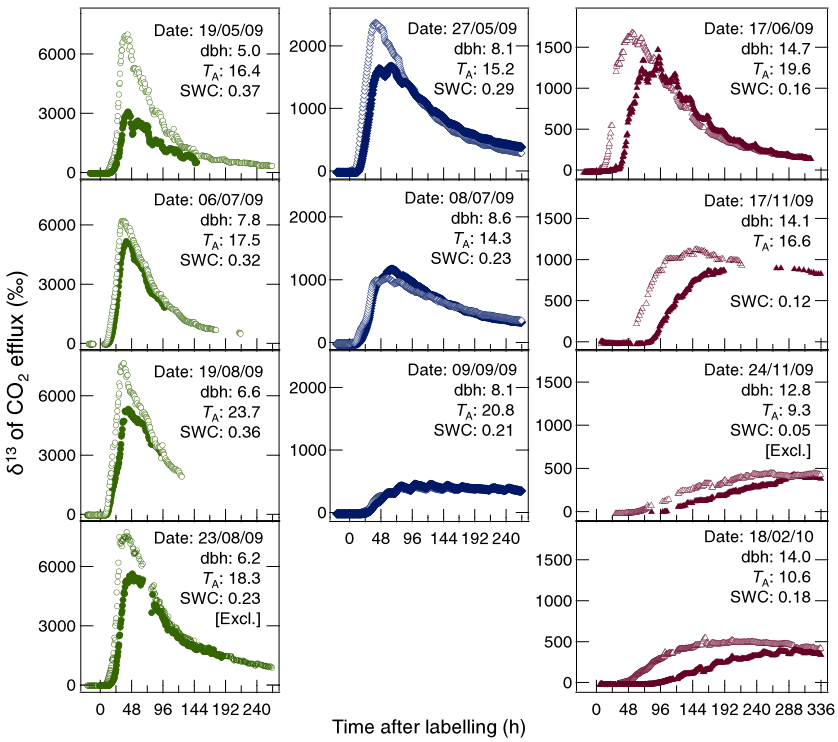

Fig. 2 Typical examples of time courses of the isotope composition of CO_2 effluxes ($\delta^{13}C_F$) in the trunk at the base of the crown (open symbols) and at the base of the trunk (closed symbols) after pulse labelling of beech (circles, left panels), oak (diamonds, central panels) and pine (triangles, right panels). The date of labelling, trunk diameter at 1.30 m height (dbh; cm), air temperature averaged for the 24 h following labelling (T_A; °C) and the volumetric soil water content (SWC; m^3 H_2O m^{-3}) are given inside each panel. 'Excl.' indicates that the tree was under a rainfall exclusion roof.

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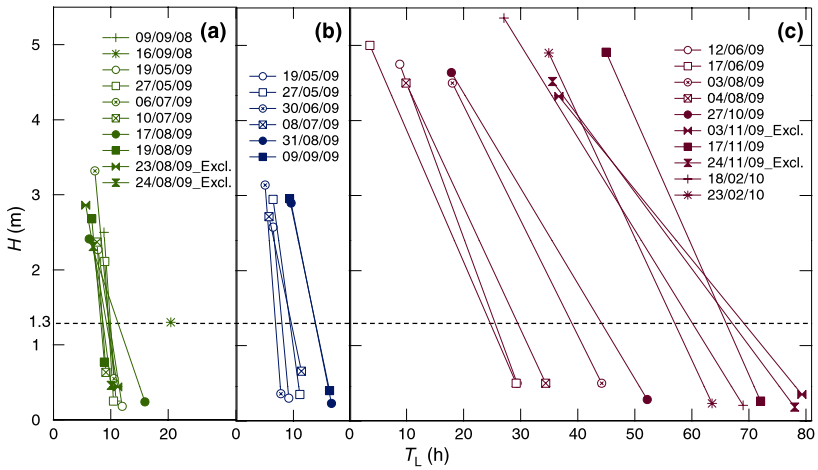

Fig. 3 Relationships between time lag from the start of the labelling to the first appearance of ^{13}C in CO_2 efflux (L_T; h) and height of the chamber above soil level (H; m). Each line represents a different tree of beech (a), oak (b) and pine (c). The horizontal dashed line indicates the position of bark sampling.

vs 55 h) and 40 h in pine during the growing season (June and August labelling). The average delay was 90 h for pine trees labelled in November and February (data not shown).

Velocity of carbon transfer

The velocity of carbon transfer in the trunk (V; m h^{-1}) exhibited both marked differences among species (slopes in Fig. 3; Table S1) and high variability within a species. Values fell within similar ranges in beech and oak, between 0.22 and 1.21 m h^{-1} in beech (mean value 0.76 m h^{-1}) and between 0.36 and 1.02 m h^{-1} in oak (mean value 0.59 m h^{-1}), and were four to five times lower in pine (between 0.09 to 0.21 m h^{-1}; mean value 0.15 m h^{-1}).

No correlation between tree size (dbh) and V was observed in pine, while there was a positive correlation in beech (R^2 = 0.66, P = 0.008; Fig. 4). A similar but not statistically significant relationship was found for oak (R^2 = 0.52, P = 0.11), and was improved by adding SWC to the model (R^2 = 0.82, P = 0.07).

V and L_{T1} were positively related to air temperature (T_A) in pine. The highest R^2 values (Fig. 5a, Table 1; Eqn M1 for

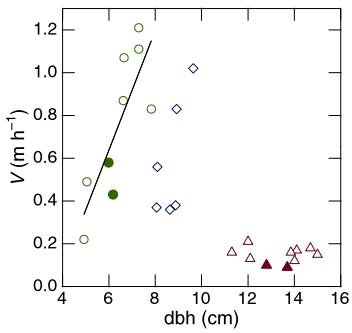

Fig. 4 Relationships between the velocity of carbon transfer in the trunk (V; m h^{-1}) and trunk diameter at 1.30 m height (dbh; cm) in beech (circles), oak (diamonds) and pine (triangles). Closed symbols are for trees under rainfall exclusion roofs. The linear regression was significant for beech (R^2 = 0.66, n = 9, P < 0.05; full line).

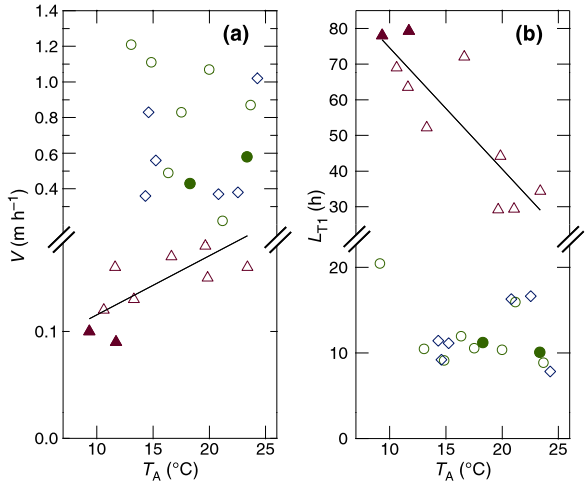

Fig. 5 Relationships between air temperature averaged for the 24 h following labelling (T_A; °C) and the velocity of carbon transfer in the trunk (V; m h^{-1}) (a) and the time lag in carbon transfer at the base of the trunk (L_{T1}; h) (b) for beech (circles), oak (diamonds) and pine (triangles). The closed symbols indicate the two trees that were under rainfall exclusion roofs. The linear regressions were significant for pine (R^2 = 0.56 and 0.74, respectively; n = 10, P < 0.05; full line). Note that the scale of the y-axes changes at V = 0.2 m h^{-1} and L_{T1} = 25 h.

V; Fig. 5b, Table 1; Eqn M2 for L_{T1}) were obtained when air temperatures were averaged during the 24 h following labelling. The relationship between V and T_A was further improved by adding SWC as a co-variable to the model (Table 1; Eqn M3). Whereas no relationship between V and T_A or between V and SWC was found for oak or beech, the bivariate model predicted V satisfactorily in oak with temperature average over 48 h (Table 1; Eqn M5), but not in beech.

Phloem sap extracts

The carbon isotope composition of phloem extracts ($\delta^{13}C_P$) collected at a height of 1.3 m increased sharply after a time

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Table 1 Velocity of carbon transfer in the trunk (V; m h^{-1}), the time lag between the start of the labelling and the first appearance of ^{13}C in trunk CO_2 efflux at the base of the trunk (L_{T1}; h) or in phloem extracts collected at 1.3 m height (L_P; h), and rate constant of the outflux of label from phloem extracts (k_P; h^{-1}) as a function of soil water content (SWC; m^3 H_2O m^{-3}) and air temperature (T_A; °C) averaged for 24 h (pine) or 48 h (oak) following labelling

Eqn	Species	Equation	Statistics
M1	Pine	$V = 0.0056^* T_A + 0.060^\dagger$	$R^2 = 0.56$, $P = 0.013$
M2	Pine	$L_{T1} = -3.40^* T_A + 109^*$	$R^2 = 0.74$, $P = 0.002$
M3	Pine	$V = 0.0055^* T_A + 0.29^\dagger \text{SWC} - 0.029$	$R^2 = 0.73$, $P = 0.011$
M4	Pine	$L_P = -3.89^* T_A + 110^*$	$R^2 = 0.58$, $P = 0.011$
M5	Oak	$V = 0.069^* T_A + 6.28^* \text{SWC} - 2.23^*$	$R^2 = 0.93$, $P = 0.017$
M6	Oak	$k_P = -0.085^* \text{SWC} + 0.048^*$	$R^2 = 0.80$, $P = 0.017$

* and † indicate that the parameter is different from zero at, respectively, $P < 0.05$ and $P < 0.1$.

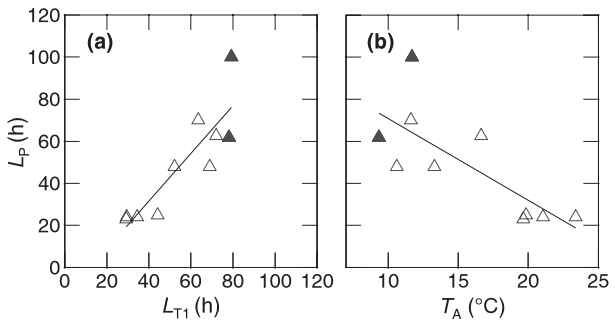

Fig. 6 Relationships between the time lag in phloem extract (L_P; h) and air temperature averaged during the 24 h following labelling (T_A; °C) (a) and the time lag in carbon transfer at the base of the trunk (L_{T1}; h) for pine (b). The closed triangles indicate the two trees that were under rainfall exclusion roofs. The linear regressions were significant (R^2 = 0.58 and 0.77, respectively, n = 10, $P < 0.05$, full line).

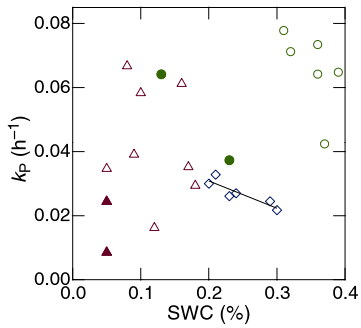

Fig. 7 Relationships between the rate constant of the outflux of label from the phloem extract (k_P; h^{-1}) and the volumetric soil water content (SWC; m^3 H_2O m^{-3}) for beech (circles), oak (diamonds) and pine (triangles). The closed symbols indicate the two trees that were under rainfall exclusion roofs. The linear regression was significant for oak (R^2 = 0.80, n = 6, $P < 0.05$; full line).

lag and started to decrease thereafter (Fig. 1b). The determination of the time lag (L_P) and the peak time was constrained by the sampling schedule, especially for beech and oak, because $\delta^{13}C_P$ started to decrease before the first sampling date in most cases. L_P values were indeed always < 1 d in beech and oak, in agreement with the time lag of $\delta^{13}C$ in CO_2 efflux at the base of the trunk (L_{T1}), except for the beech tree labelled in September 2008, when the temperature was 9°C (L_P = 37 h). L_P ranged between 23 and 100 h in pine and was closely related to L_{T1} (R^2 = 0.77, $P < 0.001$; Fig. 6a). L_P was on average 5 h less than L_{T1} in pine, which makes sense considering that phloem was collected at *c.* 1.3 m height while the bottom respiration chamber was at *c.* 0.3–0.5 m height. L_P was well correlated with air temperature in pine (Fig. 6b, Table 1; Eqn M4).

Interestingly, and despite differences in V, the rate constants of the label outflux from phloem extracts (k_P) were similar in beech and pine, ranging from 0.017 to 0.078 h^{-1} in beech (MRT between 13 and 58 h; Table S1) and from 0.009 to 0.067 h^{-1} in pine over the whole season, with the lowest values in winter (MRT between 15 and 117 h). By contrast, k_P was two times lower in oak, ranging from 0.022 to 0.033 (MRT between 30 and 46 h), and was correlated with SWC (Fig. 7, Table 1; Eqn M6).

Bark dimensions

The inner bark tissues were significantly thicker in oak (4.9 mm) and thinner in beech (2.0 mm) compared with pine (2.9 mm), and positive correlations between inner bark thickness and dbh were observed in beech (R^2 = 0.86, P < 0.001) and in oak (R^2 = 0.32, P = 0.06), but not in pine (Fig. 8). There were also significant differences in the dry mass of inner bark tissue (M_B), the dry mass of phloem sap extracts (M_E) and phloem sap extracts per unit dry mass of inner bark tissue (M_B/M_E) among the three species (Table 2).

Discussion

Time lag and velocity

The time lag between the start of the labelling and the first appearance of ^{13}C in CO_2 efflux varied with the position of the chamber along the trunk, and among trees depending on both the species and the season. It depends on the path length

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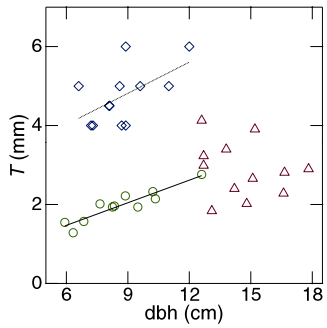

Fig. 8 Relationships between phloem thickness (T; mm) and trunk diameter at 1.30 m height (dbh; cm) in beech (circles; R^2 = 0.86), oak (diamonds; R^2 = 0.32) and pine (triangles). The linear regression was significant for beech (R^2 = 0.86, n = 11, P < 0.05; full line) and marginally significant for oak (R^2 = 0.31, n = 12, P = 0.06; dashed line).

Table 2 Dry mass of phloem tissue (M_B), phloem sap (M_E) and sap content (M_B/M_E) in beech, oak and pine

Species	M_B (g m^{-2})	M_E (g m^{-2})	M_B/M_E (mg g^{-1})
Beech (n = 168)	815 ± 233	19 ± 8	24 ± 9
Oak (n = 144)	1526 ± 493	68 ± 27	46 ± 16
Pine (n = 140)	566 ± 128	44 ± 13	80 ± 26
Species (df = 2)	1101***	487***	457***
Tree, nested within species (df = 31)	33***	10***	4***

Values are means ± SD. Analyses of variance (F ratio) for these three variables as affected by species and trees nested within species are given.
df, degree of freedom. F ratios followed by *** are significant at 0.001.

and the velocity of phloem sap, but the relationship between time lags and velocity may be more complicated if there is leakage and reloading with transitory storage and further 2remobilization of the stored carbohydrates (Minchin & Lacointe, 2005; Gessler *et al.*, 2008; Bahn *et al.*, 2009; Kuzyakov & Gavrichkova, 2010; Mencuccini & Hölttä, 2010). Indeed, the recovery of label in trunk CO_2 efflux demonstrates this leakage, which sustains trunk cell metabolism. In addition, the difference in maximum $\delta^{13}C_F$ between the base of the crown and the base of the trunk that was observed on some occasions indicates that storage or growth removes a fraction of ^{13}C transport by the phloem sap. Nevertheless, assuming similar leakage and reloading velocity at any position along the trunk, more reliable estimates of phloem sap velocity can be computed from differences in time lag at different heights.

Velocity calculated on time lags represents the maximal velocity (Fisher, 1990) and differs from average velocity depending on the radial velocity profile, both within the sieve tubes (parabolic profile according to the Poiseuille law) and in the bark. Assuming a first-order kinetics for a solute flowing out of phloem, the rate constant (k_P) is related to the average velocity (V_{avg}) and to the path length (l):

$$V_{avg} = k_P \times l$$

As expected, the average velocities computed from k_P values were lower for beech and oak than the maximal velocities computed from the time lags of labelled recovery, assuming the path length is equal to the tree height (i.e. 10 m). The difference increased with increasing maximal velocity and was more pronounced in oak than in beech. In pine, V_{avg} was unexpectedly two times higher than V. The reason for this discrepancy remains unclear. The comparison of V and V_{avg} computed from k_P estimated from sap collected at 1.3 m height assumes constant phloem and phloem sap properties along the trunk (viscosity, solute concentration, turgor pressure difference and anatomy), which may not be a valid assumption (Mencuccini & Hölttä, 2010). It has indeed been postulated that phloem diameter changes vertically (Hölttä *et al.*, 2009).

Differences among seasons

Seasonal changes in air temperature did not account for the variability of L_{T1} and V in beech, except for the late labelling in September 2008, when the air temperature was below 10°C. The results for beech contrasted with those obtained for oak and pine, for which V could be accurately predicted by a bivariate linear regression with T_A and SWC. While temperature may affect active processes such as phloem loading, the mechanism by which the temperature may influence phloem transport velocities is still unclear. A temporary inhibition of phloem sap velocity by petiole cooling was observed in sugar beet (*Beta vulgaris*) and bean (*Phaseolus vulgaris*) plants (Geiger & Sovonick, 1970). Theoretical analysis of phloem transport has shown that the maximum velocity is limited by solution viscosity (Hölttä *et al.*, 2009). A drop in temperature will increase the viscosity of the phloem sap and will decrease its velocity. The decrease in velocity might be reinforced by a blockage of sieve plates by P proteins (Peuke *et al.*, 2006). Imbalance between phloem loading and unloading might also change the gradient of hydrostatic pressure along the phloem.

The hydrostatic pressure difference between source (phloem loading) and sink (phloem unloading) organs, and the subsequent exchange of water between sieve cells and the apoplast, is thought to drive phloem transport of assimilates according to the Münch theory (Hölttä *et al.*, 2009). Consistently, sap velocities measured in the phloem are of the same order of magnitude as those found in the xylem (Helfter *et al.*, 2007). This close coupling between xylem and phloem transport makes it likely that a change in plant water potential will affect the rate of phloem transport (Hölttä *et al.*, 2009). A depressive effect of water stress on carbohydrate transport in the phloem was indeed observed in water-stressed beech seedlings, for

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which the build-up of ^{13}C in twig phloem sap after pulse labelling was slower than in control seedlings (Ruehr *et al.*, 2009). These authors also observed that ^{13}C peaked 2 d later in the trunk phloem compared with the twig phloem, suggesting a velocity of 0.01 m h^{-1}. In contrast to these results, there was no relationship between SWC and either L_{T1}, V, L_P or k_P for beech in our study. This might be attributable to a much milder drought treatment in this experiment than with the potted seedlings, as predawn leaf water potential values were similar for the four trees labelled in August (−0.4 MPa; data not shown) despite the difference in SWC. In oak and pine, SWC, together with temperature, affects the velocity of C transfer in the trunk (V). The predawn leaf water potential of oak dropped below −0.9 MPa in early September. An imbalance between phloem loading (which might decrease with a drought-induced decrease in net CO_2 assimilation) and unloading, as well as a reduction in transpiration as a result of stomatal closure, should decrease the gradient of hydrostatic pressure along the phloem and thus transport velocity. However, the rate constant of carbon outflux in the phloem (k_P) was unexpectedly negatively related to SWC in oak, indicating opposite trends for V and V_{avg} computed with k_P estimated from sap collected at 1.3 m height.

Differences among species

Velocity differences between pine and the two broadleaf species are consistent with previous measurements on young seedlings. A value of 0.12 m h^{-1} was previously reported for *Pinus Banksiana* and *Picea abies* seedlings, while values ranging between 0.3 and 0.6 m h^{-1} were reported for Fraxinus pennsylvanica ash and *Ulmus americana* using ^{11}C labelling (Thompson *et al.*, 1979). The slower rate in coniferous compared with broadleaf trees is consistent with recent laboratory experiments showing a delayed peak of ^{14}C recovery in belowground CO_2 efflux in *Pinus sylvestris* and *Picea abies* Norway compared with *Betula pendula* seedlings (Pumpanen *et al.*, 2009). However, such results contrast with indirect values derived from time-lagged correlations between the ^{18}O compositions of phloem sap at different heights in the trunk of 38-yr-old *Pinus sylvestris* (Barnard *et al.*, 2007), which were about five times higher (0.5–1 m h^{-1}) than those we calculated for *P. pinaster*.

Differences in phloem anatomy may account for differences in phloem transport rates between coniferous and broadleaf trees. According to Poiseuille's law, the velocity will depend on the gradient of hydrostatic pressure along the phloem, on the length of the path between source and sink organs and on the hydraulic conductivity (K). K increases by the power of 2 as the tube radius increases (r) and decreases with increasing fluid viscosity (η; i.e. with increasing sugar concentration or decreasing temperature). In addition, the anatomy of the sieve plate is also important. K will decrease with increasing pore length or with decreasing pore radius or number of pores (Sheehy *et al.*, 1995; Thompson, 2006). Most of the resistance to sap flow is attributable to the pore dimension (Thompson & Holbrook, 2003) and may be additionally restricted by callose formation (Mullendore *et al.*, 2010). A higher diameter of sieve tube elements (20–50 μm; Trockenbrodt, 1994; Sheehy *et al.*, 1995) than of sieve cells (10–30 μm for 16 pine species; Shigematsu, 1959) and a better connection at the sieve plate between sieve tube elements than between sieve cells (Hepton & Preston, 1960; Parthasarathy, 1975) are therefore thought to account for the higher conductivity in the two broadleaf species than in pine, and, for a given gradient of hydrostatic pressure, to the higher velocity of phloem sap. Information about gradients of hydrostatic pressure in the phloem of trees is rather scarce (Milburn, 1975). Values ranging between 0.02 and 0.06 MPa m^{-1} were reported for oak (Hammel, 1968) and a value of 0.05 MPa m^{-1} is commonly assumed for trees (Milburn, 1975).

The amount of photosynthates transported by the phloem sap depends also on the phloem sap concentration (Canny, 1975). Despite similar velocities for oak and beech, the mass flow density, that is, the product of velocity by sap concentration, is higher in oak than in beech, and provided that the sap content, when expressed in mg g^{-1}, is a good proxy for sap concentration. Because the inner bark (phloem tissue) of oak is twice as thick as that of beech, the total mass flow may be much higher in oak than in beech. However, the functional secondary phloem is smaller than the bark thickness, the proportion of sieve elements in the phloem varying between 20 and 70% of the cross-sectional area (Curtis, 1936; Canny, 1975). In addition, the phloem tissue produced in previous years may not be functional in some species, the layer of conducting phloem ranging from 0.2 to 1 mm only in broadleaf trees (Sheehy *et al.*, 1995; Kozlowski & Pallardy, 1997), but comparative anatomy of oak and beech phloem is lacking.

Effects of tree dimension

The independence between dbh and V observed in pine suggests that the product of V and bark cross-sectional area (i.e. the volumetric flow in m^3 s^{-1}) increases linearly with increasing phloem area, probably scaling with increasing crown photosynthesis. The number of replicates was too small to show an eventual relationship between dbh and V in oak. Such relationships might have been hidden by a drop in water potential in August, as suggested by a positive relationship between dbh and V observed when SWC is added as a co-variable to the model. The positive relationship between dbh and V in beech, which is only partly offset by the increase in bark thickness with dbh, indicates that the volumetric flow increases as a power of bark cross-sectional area > 1. This suggests changes in allocation pattern with tree size in this species, that is, an increase in carbon allocation belowground with increasing tree size. The negative correlation between dbh and the

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difference in maximum $\delta^{13}C_F$ between the base of the crown and the base of the trunk also suggests that less carbon is removed from the phloem sap between the crown and the soil.

Conclusions

The vertical velocity of carbon transfer in trees 8–10 m tall differed markedly between two deciduous broadleaf species (beech and oak) and maritime pine, reflecting difference in phloem anatomy between angiosperms and gymnosperms, but little is known about species differences in the functional anatomy of phloem. The velocity was positively related to tree diameter in the two broadleaf species, suggesting that the volumetric flow increased more than the phloem cross-sectional area with increasing tree diameter. The velocity of carbon transfer changed seasonally according to changes in temperature and soil water content in oak and in pine, but the mechanism behind this is still unclear and additional experiments are required to test the hypothesis that an imbalance between phloem loading (source activity) and unloading (sink demand) may account for variation in the velocity. A better understanding of this mechanism will be a prerequisite for coupling phloem transfer to leaf photosynthesis and to organ growth and respiration in forest ecosystem models.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Values of environmental variables and estimated parameters for all labelled trees

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Research paper

Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees

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We assessed the pools of non-structural nitrogen compounds (NSNC) through a year, thereby addressing the question of whether mature sessile oak [*Quercus petraea* (Matt.) Liebl.] and beech (*Fagus sylvatica* L.), which differ in wood anatomy and growth patterns, exhibit contrasting seasonal dynamics of NSNC pools as previously shown for non-structural carbohydrate (NSC) pools. Seasonal fluctuations of NSNC (amino acids and soluble proteins) and NSC (starch and soluble sugars) pools were analyzed in the inner and the outer stem sapwood. In oak, NSC showed marked seasonal variation within the stem sapwood (accumulation during winter and decrease during bud burst and early wood growth), whereas in beech seasonal fluctuations in NSC were of minor amplitude. Even if the distribution and intensity of the NSNC pools differed between the two species, NSNC of the stem sapwood did not show seasonal variation. The most significant change in NSNC pools was the seasonal fluctuation of protein composition. In both species, two polypeptides of 13 kDa (PP13) and 26 kDa (PP26) accumulated during the coldest period in parallel with starch to sugar conversion and disappeared with the onset of spring growth. The absence of seasonal changes in total soluble protein concentration suggests that the polypeptides are involved in the internal nitrogen (N) cycling of the stem rather than in N storage and remobilization to the other growing organs of the tree.

Keywords: *Fagus sylvatica* L., non-structural carbohydrates, non-structural nitrogen compounds, phenology, polypeptides, *Quercus petraea* (Matt.) Liebl., stem sapwood.

Introduction

Non-structural carbohydrate (NSC) and nitrogenous compounds (NSNC) are known to play an essential role during winter by supplying carbon (C) and nitrogen (N) for maintenance metabolism and during early spring for new growth of deciduous tree species (Kramer and Kozlowski 1979, Stepien et al. 1994, Sauter and Witt 1997). However, detailed investigations of the seasonal dynamics of these compounds in adult broad-leaved temperate forest trees, in relation to their natural environment, are more detailed for NSC (Piispanen and Saranpää 2001, Barbaroux and Bréda 2002, Hoch et al. 2003) than for NSNC. In fact, seasonal dynamics of NSNC such as amino acids and/or vegetative storage proteins (VSPs) have been mainly assessed in young poplar (Stepien et al. 1994, Black et al. 2001, Cooke and Weih 2005, Rennenberg et al. 2010), tropical tree species (Tian et al. 2003) and fruit trees (O'Kennedy and Titus 1979, Gomez and Faurobert 2002, Wisniewski et al. 2004) whereas data are scarce for adult forest trees. As N can be a growth-limiting factor in natural forest ecosystems (Rennenberg et al. 1998), the ability of forest trees to store and redistribute N resources internally is a fundamental process conditioning both C and N budgets (Millard and Grelet 2010). Studies comparing both C and N internal cycles during the life span of forest tree species are lacking and, to our knowledge, the literature mainly concerns tree saplings (Cerasoli et al. 2004*a*, 2004*b*, Vizoso et al. 2008).

When mature trees were studied, the analysis of N pools was commonly restricted to either bark or wood of young organs (Wetzel and Greenwood 1991, Wisniewski et al. 2004) but rarely to stem sapwood whereas NSC storage in this compartment

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was well characterized in several temperate tree species (Hoch et al. 2003). Barbaroux et al. (2003) showed that stem sapwood was quantitatively the main C-storage compartment in adult oak and beech trees and that stored NSC provide a large part of the C required for winter maintenance respiration and for spring growth in adult oak, and to a lesser extent in adult beech.

Sessile oak and beech are two of the most widespread temperate broadleaved species in the north-east of Europe. They differ in wood anatomy (Gasson 1987) and exhibit contrasting growth patterns. In oak, a ring-porous species, the tree achieves earlywood growth before leaf expansion in spring and C autotrophy (Dougherty et al. 1979, Hinckley and Lassoie 1981, Bréda and Granier 1996). By contrast, in beech, a diffuse-porous species, stem growth begins after leaf expansion. In the stem sapwood of these species, Barbaroux and Bréda (2002) showed contrasting seasonal dynamics of NSC, in agreement with the contrasting growth pattern of these species and a different radial distribution of NSC based on ring age for the two species. In oak, NSC were restricted to the sapwood rings and were highly mobilized during spring, whereas in beech, NSC were distributed throughout the wood from the outermost sapwood ring to the pith and spring growth was less dependent on stored C. These dynamics reflected differences in the management of C sources (reserves, photoassimilates) by the two species.

In the present study, we aimed to describe the seasonal course of NSNC in the inner and the outer stem sapwood of mature oak and beech trees. We assessed NSNC pools through a year, thereby addressing the question of whether these species, which differ in wood anatomy and growth patterns, exhibit contrasting seasonal dynamics of NSNC pools as previously shown for NSC pools. Although the dynamics of C pools have already been studied in trees of both species originating from the same forest sites (Barbaroux and Bréda 2002, Barbaroux et al. 2003), the trees analyzed in the present study were 10 years older. Here, we compared the seasonal fluctuation of NSNC (amino acids and soluble proteins) with that of NSC (starch and soluble sugars) in the inner and the outer stem sapwood of 50-year-old oak and beech trees. In addition, the seasonal dynamics of soluble protein composition were analyzed in order to identify some specific proteins whose function will be discussed in relation to phenology and climatic conditions.

Materials and methods

Study site

The study was conducted in a pure stand of sessile oak [*Quercus petraea* (Matt.) Liebl.] in the state forest of Champenoux, France (48°44′ N, 6°14′ E, elevation 237 m) and in an almost pure stand of common beech (*Fagus sylvatica* L.) in the state forest of Hesse, France (48°40′ N, 7°05′ E, elevation 300 m), both from natural regeneration. In 2008, oak and beech trees were 50–55 and 45–50 years old, respectively. All the standard climatic variables were measured in weather stations monitored by INRA installed close to the study sites. Daily minimum and maximum air temperatures were calculated from hourly data during the study period. Mean annual precipitation was 744 and 820 mm, respectively. Stand characteristics of both sites are given in Table 1; for additional details on soil type, see Bréda et al. (1995) and Granier et al. (2000). Four dominant or co-dominant trees of each species were selected for the experiment. Mean stem diameter at breast height was 17 and 22 cm for beech and oak, respectively.

Bud burst and stem increment monitoring

Bud burst was recorded every 2–3 days from the beginning of April to the end of May on the same trees. Bud development was described according to a six-stage scale (dormant winter buds, swollen buds, broken buds, just-unfolded leaves, unfolded leaves and developed leaves with shoot elongation). Bud burst was achieved when the tree reached the unfolded leaf stage (Bréda and Granier 1996), which corresponded to the onset of both leaf expansion and increase in leaf area index. Stem radial increment was measured on all trees every 5 days with dendrometer bands anchored at 1.3 m height.

Wood sampling

One core (5 mm diameter and 10 cm long) was sampled on each tree every month from October 2007 to October 2008. The first sample was taken in the northerly direction and the following monthly cores were shifted a few centimeters to the left of the previous one and thus distributed in a spiral along the stem circumference at 1.3 m height. Previous measurements in

Table 1. Main stand characteristics of the two experimental sites, Champenoux and Hesse. DBH, diameter at breast height; LAI, leaf area index.

	Champenoux	Hesse
Location	48°44′ N, 6°14′ E	48°40′ N, 7°05′ E
Elevation (m)	237	300
Mean annual precipitation (mm)	744	820
Mean annual temperature (°C)	9.2	9.2
Species	*Q. petraea* (Matt.) Liebl.	*F. sylvatica* L.
Age, years	50–55	45–50
Stand density (stems ha^{-1})	2553	2600
Basal area (m^2 ha^{-1})	26.7	20.8
Mean DBH (cm)	22	17
Maximum LAI	5.6	8.7
Soil type (FAO classification)	Cambisol[1]	Cambisol dystric[2]
Humus type	oligo-mull	oligo-mull
Soil N content (Horizon A1, g kg^{-1})	2.14	0.29
Soil C/N	13.5	16.2

For details see [1]Bréda et al. (1995) and [2]Granier et al. (2000).

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these two stands have shown no effect of stem orientation on carbohydrate concentration (Barbaroux and Bréda 2002). Samples were conserved in liquid N in the field and then stored at –80 °C (–86 °C Freezer® Forma Scientific®, Marietta, OH, USA) until freeze-drying (Dura-Top®, Dura-Dry® FTS Systems®, Stone Ridge, NY, USA). Bark was removed from each sampled core. As the previous 'ring by ring' study by Barbaroux and Bréda (2002) showed a sharp radial decrease in carbohydrate content toward the pith, sapwood was divided into two compartments according to ring age: the 2 most recent rings (outermost sapwood designated 'RR' in the study) and the 8–10 older rings (innermost sapwood designated 'OR'). All samples were weighed, finely ground (Mixer Mill MM200, Retsch, Germany) and then stored in the dark in airtight vials for biochemical analyses.

NSC analyses

Soluble sugars were extracted from 15 (RR) to 20 mg (OR) of dry matter (DM) of each sapwood compartment with 1 ml of 70% (v/v) methanol/water for 10 min and then centrifuged at 17,000*g* for 5 min at 4 °C. This step was repeated twice and the resulting supernatants were pooled together. The pellet was retained for starch analysis and the supernatants, containing soluble sugars, were dried overnight with a vacuum evaporator (Maxi-Dry Plus; Hetomodel DW1, 0-110, Heto-HOLTEN A/S Allerod, Denmark) to eliminate methanol. Dried extracts were rehydrated in 1.5 ml of distilled water, redissolved by sonication and then filtered at 0.2 μm (Acrodisc® Supor® filter; 0.2 μm, Van Waters Rogers). Undiluted aliquots of 30 μl were injected into a high-pressure liquid chromatography system (Gold®Beckman Coulter, Software 32 karat™) to determine soluble sugar concentration and composition. Soluble sugar separation was achieved on a HyperRez XP Carbohydrate Pb^{2+} separation column (8 μm 300 × 7.7 mm; Thermo Electron, Courtaboeuf, France) fitted with a precolumn (HyperRez Pb^{2+} 5 × 3 mm; Thermo Electron). The flow rate was maintained at 0.6 ml min^{-1}, the pressure between 6.8×10^6 and 10×10^6 Pa and the column temperature at 83 °C. Individual carbohydrates were eluted from 0 to 30 min after injection. The sugar peaks were detected by light scattering (Sedex 45 ELSD system, Seder, Vitry sur Seine, France). Peak identification was determined according to the retention time of commercial carbohydrate standards. Sucrose, glucose, fructose and mannose were the main sugars detected. Sugar concentrations were determined by peak height and standard calibrations.

The pellet was analyzed for starch concentration by hydrolysis with 0.02 N NaOH for 30 min at 100 °C followed by digestion with amyloglucosidase (from *Aspergillus niger*, EC 232-877-2, Sigma Aldrich, Saint Quentin Fallavier, France) for 30 min at 50 °C in order to hydrolyze starch into glucose molecules. Glucose was quantified using hexokinase and glucose-6-phosphate dehydrogenase followed by spectrophotometric determination of NADPH formation at 340 nm (Boehringer 1984).

Soluble sugar and starch concentrations were expressed on a DM basis (% DM). Their sum was named NSC.

NSNC analyses

Total amino acid concentration was determined on an aliquot of 50 μl of the soluble fraction (see above) by colorimetry at 570 nm (spectrophotometer UV–visible DU 640 B, Beckman Coulter, Fullerton, CA, USA) as described by Yemm and Cocking (1955) using ninhydrin with leucine as standard.

Soluble proteins were extracted from 15 (RR) to 40 mg (OR) of DM mixed with 500 μl of extraction buffer [62.5 mM Tris HCl pH 6.8, 2% (v/v) sodium dodecyl sulfate (SDS), 10% glycerol and 28 mM dithiothreitol (DTT)] in a micro-tube, using a ball mill (Retsch MM 301, Gmbh & Co, Germany) twice for 45 s. Samples were centrifuged twice at 32,000*g* for 20 min (17 °C). Soluble proteins were precipitated with 4 volumes of 10% trichloroacetic acid in cold acetone and 28 mM DTT and stored at –20 °C overnight. The following day, proteins were collected by centrifuging at 16,000*g*, 4 °C for 10 min, and then washed four times with 1.5 ml of cold acetone and 28 mM DTT. After each washing, the mixture was stored for 30 min at –20 °C, and then centrifuged at 16,000*g*, 4 °C for 15 min. The final pellet was air-dried at room temperature (30 min) and then solubilized in 15 μl of Laemmli buffer (62.5 M Tris–HCl pH 6.8, 2 M SDS, 10 M glycerol, 28 mM DTT and 5 mM bromophenol blue). Protein concentration from the solubilized pellet was determined by RC-DC Protein Assay (Bio-Rad RC DC Protein Assay 500-0121). The remaining pellet was boiled for 5 min and then cooled at room temperature.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described by Laemmli (1970) with a Mini-Protean II electrophoretic unit (Bio-Rad) using a 4% stacking gel and a 12.5% running SDS–PAGE. Proteins from 10 and 25 mg of DM were loaded in each lane for RR and OR, respectively. Gels were stained with 0.1% (w/v) Coomassie Brilliant Blue G-250 and destained with 25% (v/v) ethanol and 7% (v/v) acetic acid. Gels were digitized with a Bio-Rad model GS 690 Imaging densitometer scanner. The integrated intensity of protein bands was measured manually using Bio-Rad Multi-Analyst software (Quantity One®) and then plotted relative to the total intensity of the proteins in the lane. Each SDS–PAGE corresponds to protein extracts from one compartment of a single tree sampled at one of the 13 dates. PAGE was performed on cores collected on two of the sampled trees. Correct repeatability was observed at most time points in sapwood compartment and species.

Amino acid and soluble protein concentrations were expressed on a DM basis (% DM). Their sum was named NSNC.

Statistics

Separate analyses were performed in each species using a mixed effects linear model, with date (13 levels), compartment

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(two levels: RR and OR) and their interaction as fixed effects. A tree random effect and possibly a tree × compartment interaction random effect were introduced in models to account for pseudoreplicates. When heteroscedasticity was present, variance was modeled as a power function of the predicted one. The best combination of random effects and variance models was selected according to log-likelihood ratio tests with the same fixed effects model. Post hoc tests were then performed to compare dates within each compartment and compartments within each date. Some sugar concentrations (glucose and fructose in beech) were mostly zero over several months, which violated the normality assumption of residuals and then the models above were applied only on dates (October to April) with mostly non-zero concentrations (Table 2).

All analyses were performed with *R* version 2.12.2 (R Development Core Team 2011). Mixed models were fitted with the *lme* function of R core package *nlme* version 3.1-98 (Pinheiro et al. 2011). Post hoc tests with control of familywise error rate were performed with the *glht* function of *multcomp* version 1.2-5 R package (Hothorn et al. 2008).

Results

Daily mean air temperatures

Between November 2007 and April 2008, daily minimum air temperatures were frequently <0 °C in both sites (Figures 1a and 2a). The coldest period occurred between mid-December 2007 and the end of March 2008 in both sites. The coldest daily minimum air temperature was registered on 22 December, 2007 at Champenoux (−11.5 °C) and on 25 December 2007 at Hesse (−10.1 °C). The coldest period lasted from 14 to 29 December 2007. From April, both minimum and maximum daily mean air temperatures increased and reached their highest values between July and August 2008 in both sites. The highest daily air temperature was registered on 31 July 2007 at Champenoux (Figure 1a, 30.7 °C) and at Hesse (Figure 2a, 29.5 °C). The warmest period lasted from 24 July to 7 August, 2008. Both minimum and maximum daily mean air temperatures started to decrease in September 2008 and reached lower values in October 2008 compared with October 2007.

Bud burst, leafy period and stem growth during 2008

Sessile oak and common beech differed in their growth pattern. For sessile oak (Figure 1), bud burst and stem girth increment started on 18 April. For beech (Figure 2), bud burst started on 25 April and stem growth started a month later (on 20 May) compared with oak. Bud burst was achieved by 7 May in both species, whereas stem growth persisted until the end of August. The leafy period lasted until the end of October.

Seasonal variations in NSC and NSNC in sessile oak

In RR, starch concentration was maximal at leaf fall in October 2007 and then decreased from December 2007 to February 2008 (Figure 1c, Table 3). From March to May, starch concentration increased to reach similar values to those measured in October 2007. The lowest starch concentrations were observed

Table 2. Analysis of variance of the effects of the stem sapwood compartment, date and the interaction between compartment and date on seasonal dynamics of NSNC, soluble proteins, amino acids and NSC, starch, sucrose, glucose, fructose and mannose concentrations.

Compound	Species	ANOVA (*P* value)		
		Compartment	Date	Compartment × date
NSNC	Sessile oak	0.0376	0.0358	0.0379
	Beech	0.6517	<.0001	0.0051
Soluble proteins	Sessile oak	0.1968	0.0197	0.344
	Beech	0.0941	0.0001	0.0717
Amino acids	Sessile oak	0.0177	<0.0001	<0.0001
	Beech	0.2275	<0.0001	<0.0001
NSC	Sessile oak	0.0032	<0.0001	0.0303
	Beech	0.7746	0.0008	0.005
Soluble sugars	Sessile oak	0.2106	<0.0001	0.0128
	Beech	0.8274	<0.0001	0.5034
Starch	Sessile oak	0.005	<0.0001	0.0546
	Beech	0.7576	<0.0001	0.0005
Sucrose	Sessile oak	0.15	0.0005	0.2924
	Beech	1	<0.0001	0.0537
Glucose	Sessile oak	0.794	<0.0001	<0.0001
	Beech	0.7522	<0.0001	0.8581
Fructose	Sessile oak	0.6792	<0.0001	0.0079
	Beech	0.1134	0.0001	0.5665
Mannose	Sessile oak	0.8146	0.4759	0.0001
	Beech	–	–	–

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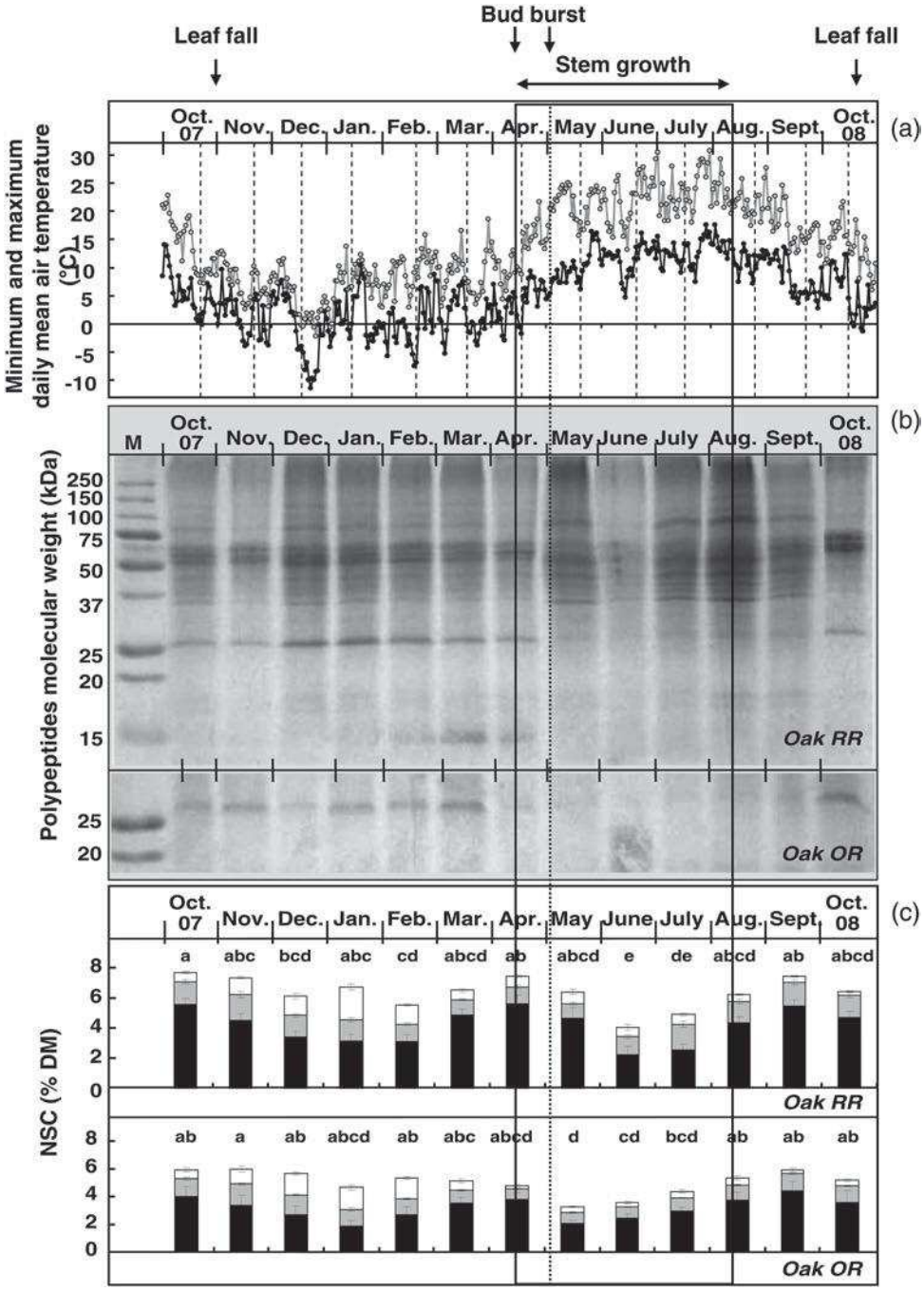

Figure 1. Sessile oak stem sapwood. (a) Minimum and maximum daily mean air temperature (°C) during the study period from October 2007 to October 2008; vertical dashed lines indicate the sampling dates. (b) Polypeptides profiles from SDS–PAGE showing the seasonal variation in soluble protein composition in the most recent rings (RR) of the stem sapwood of 50-year-old oak. For the oldest rings (OR), gels were cut to show only the seasonal variation in the 26 kDa polypeptide. For each lane, proteins from 10 mg of DM (RR) and 25 mg of DM (OR) were loaded. The molecular weights of standard polypeptides (M for markers) are shown on the left. (c) Seasonal variations in NSC concentration (% DM) in RR and OR ($n = 4$). NSC composition was starch (black), sucrose (gray) and hexoses: glucose + fructose + mannose (white). Different letters in the same line indicate significant differences between dates (at $P < 0.05$). The standard errors for starch, sucrose and hexoses are presented on the histograms. Black arrows indicate times of bud burst and leaf fall, vertical dashed lines indicate the period of bud burst and vertical black lines correspond to the period of stem radial increment.

in June and July. After these dates, starch accumulated again and reached similar values to those of October 2007. Among soluble sugars, sucrose concentrations did not display strong variations through the year and were significantly higher in November 2007 than in May 2008. Hexose concentrations increased significantly during the winter period (Figure 1). The increase in hexose concentrations resulted mainly from glucose and fructose accumulation (Table 3), whereas mannose

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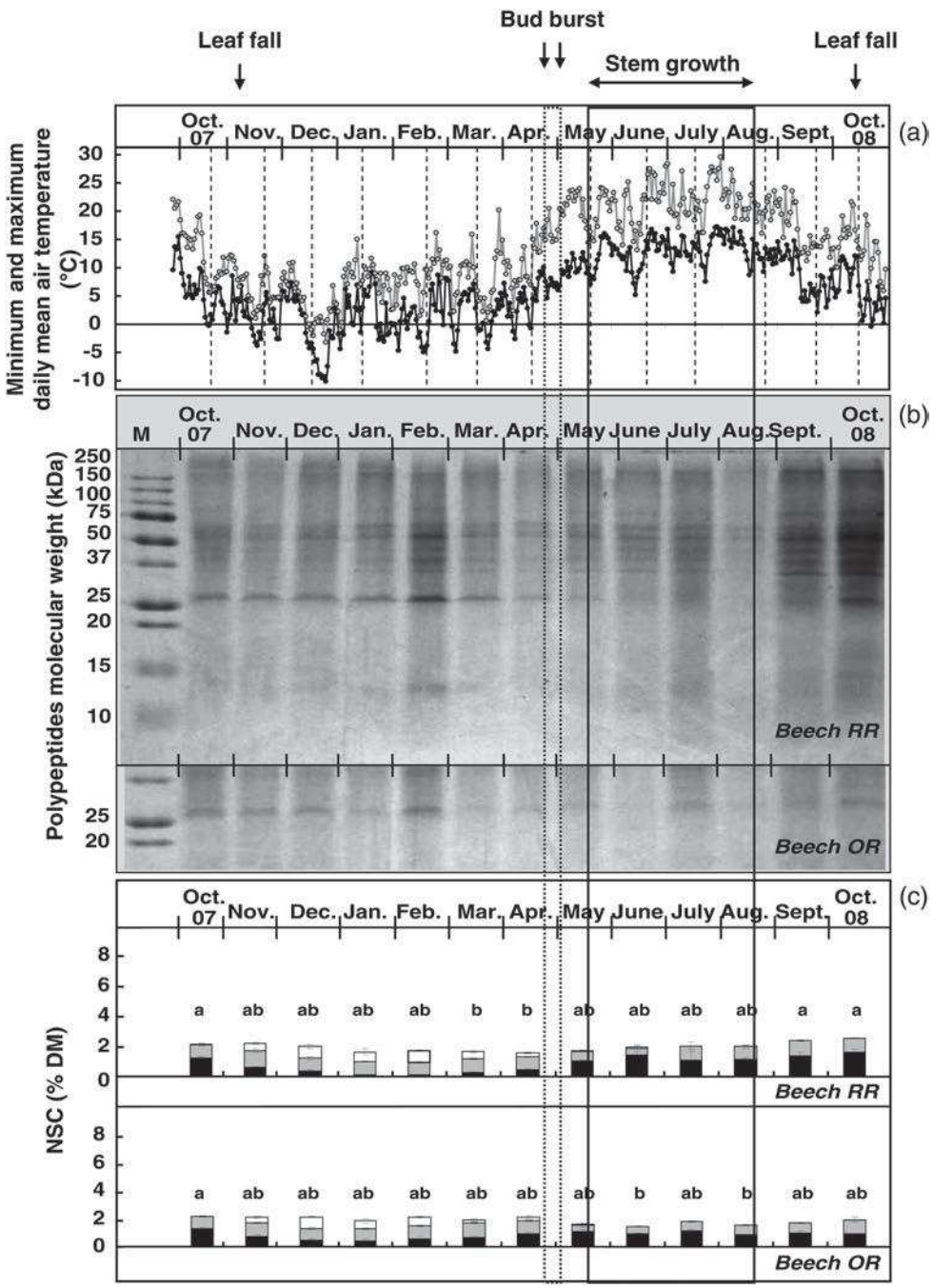

Figure 2. Beech stem sapwood. For details see legend to Figure 1.

concentration did not display any seasonal variation. Starch and sucrose concentrations were significantly lower in OR than in RR during the growth period whereas glucose and fructose concentrations were significantly lower in OR than in RR during winter.

Among NSNC, amino acid concentration displayed seasonal variations in OR only (Table 3) and was significantly lower from January to February and from March to June than in October 2007. The soluble protein concentration in RR and OR was rather stable through the year (Table 3). By contrast, the composition of soluble proteins showed annual variations, as illustrated in Figure 1b. A maximum of nine polypeptides were detected in RR and their apparent molecular weight ranged from 13 to 158 kDa (Figure 1b). A change in the overall protein patterns was observed between winter and summer. Two polypeptides of 13 kDa (PP13) and 26 kDa (PP26) accumulated during winter whereas higher molecular weight polypeptides were less abundant (PP87–PP119) or absent (PP158). The accumulation of PP26 was maximal in January and accounted for 23% of total soluble proteins in RR and for 32% in OR. The

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Table 3. Monthly NSNC (soluble proteins and free amino acids) and NSC (starch, sucrose, glucose, fructose and mannose) concentrations (% DM) in the most recent rings (RR) and oldest rings (OR) in the stem sapwood of 50-year-old *Q. petraea* (Matt.) Liebl. Different letters in the same line indicate significant differences between dates (at $P < 0.05$). Stars indicate significant differences between compartments: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Compound (% DM)	Compart-ment	October 2007	November 2007	December 2007	January 2008	February 2008	March 2008	April 2008	May 2008	June 2008	July 2008	August 2008	September 2008	October 2008
Soluble proteins	RR	0.08 ± 0.03b	0.12 ± 0.03ab	0.14 ± 0.03ab	0.11 ± 0.03ab	0.14 ± 0.03ab	0.09 ± 0.03b	0.09 ± 0.03b	0.11 ± 0.03ab	0.12 ± 0.03ab	0.17 ± 0.03a (***)	0.15 ± 0.03ab	0.09 ± 0.03ab	0.11 ± 0.03ab
	OR	0.03 ± 0.02a	0.05 ± 0.02a	0.05 ± 0.02a	0.03 ± 0.02a	0.06 ± 0.02a	0.04 ± 0.02a	0.05 ± 0.02a	0.03 ± 0.02a	0.04 ± 0.02a	0.04 ± 0.02a	0.06 ± 0.02a	0.07 ± 0.02a	0.05 ± 0.02a
Amino acids	RR	0.20 ± 0.04ab	0.26 ± 0.05a (*)	0.23 ± 0.04ab (*)	0.20 ± 0.04ab (***)	0.20 ± 0.04ab (**)	0.24 ± 0.05ab (*)	0.27 ± 0.05a (*)	0.21 ± 0.04ab (***)	0.16 ± 0.04ab (**)	0.23 ± 0.04ab (*)	0.19 ± 0.04ab (**)	0.15 ± 0.03b	0.16 ± 0.04ab
	OR	0.12 ± 0.01ab	0.11 ± 0.01abc	0.11 ± 0.01abc	0.08 ± 0.01cde	0.08 ± 0.01bcde	0.12 ± 0.01ab	0.11 ± 0.01abc	0.07 ± 0.01de	0.06 ± 0.01e	0.11 ± 0.01abcd	0.09 ± 0.01bcd	0.13 ± 0.02a	0.11 ± 0.01abc
Starch	RR	5.53 ± 0.39a	4.48 ± 0.44abc	3.38 ± 0.39bcd	3.14 ± 0.44cd	3.08 ± 0.44cd	4.81 ± 0.44ab	5.60 ± 0.52a (*)	4.61 ± 0.72abc (*)	2.23 ± 0.52d	2.52 ± 0.39d	4.33 ± 0.39abc	5.42 ± 0.39a	4.66 ± 0.44abc
	OR	3.77 ± 0.56ab	3.19 ± 0.56abcd	2.54 ± 0.56bcd	1.95 ± 0.59d	2.54 ± 0.56bcd	3.32 ± 0.56abcd	3.57 ± 0.56abc	1.99 ± 0.56d	2.32 ± 0.56cd	2.80 ± 0.56abcd	3.52 ± 0.56abc	4.18 ± 0.56a	3.38 ± 0.56abcd
Sucrose	RR	1.52 ± 0.16abc	1.69 ± 0.19a	1.42 ± 0.14abc	1.39 ± 0.14abc	1.15 ± 0.10abc	1.04 ± 0.09bc	1.08 ± 0.11abc (*)	0.98 ± 0.10c	1.19 ± 0.13abc (*)	1.72 ± 0.19a (***)	1.42 ± 0.14abc	1.55 ± 0.16ab	1.47 ± 0.15abc
	OR	1.23 ± 0.12ab	1.46 ± 0.15a	1.36 ± 0.13a	1.17 ± 0.11ab	1.09 ± 0.10abc	0.89 ± 0.07bcd	0.72 ± 0.05d	0.70 ± 0.05d	0.76 ± 0.06cd	0.90 ± 0.07bcd	1.03 ± 0.09abc	1.15 ± 0.10ab	1.12 ± 0.10ab
Glucose	RR	0.21 ± 0.05d	0.48 ± 0.05bc	0.62 ± 0.05b	1.08 ± 0.05a (***)	0.57 ± 0.05b	0.17 ± 0.05de	0.22 ± 0.06d	0.26 ± 0.06cd	0.13 ± 0.06de	0.22 ± 0.05d	0.05 ± 0.05de	0.08 ± 0.05de	0.00 ± 0.05e
	OR	0.23 ± 0.05cde	0.35 ± 0.05bc	0.53 ± 0.05a	0.65 ± 0.06a	0.47 ± 0.05ab	0.20 ± 0.05cde	0.04 ± 0.05e	0.29 ± 0.05bc	0.03 ± 0.05e	0.25 ± 0.05cd	0.22 ± 0.05cde	0.08 ± 0.05de	0.05 ± 0.05de
Fructose	RR	0.15 ± 0.06e	0.40 ± 0.06cd	0.46 ± 0.06bc	0.92 ± 0.06a (***)	0.62 ± 0.06b	0.19 ± 0.06de	0.17 ± 0.07de	0.22 ± 0.07de	0.18 ± 0.07de	0.11 ± 0.06e	0.09 ± 0.06e	0.11 ± 0.06e	0.05 ± 0.06e
	OR	0.12 ± 0.06bc	0.27 ± 0.06b	0.49 ± 0.06a	0.54 ± 0.06a	0.52 ± 0.06a	0.26 ± 0.06b	0.07 ± 0.06bc	0.07 ± 0.06bc	0.12 ± 0.06bc	0.05 ± 0.06bc	0.07 ± 0.06bc	0.03 ± 0.06c	0.09 ± 0.06bc
Mannose	RR	0.26 ± 0.07a	0.26 ± 0.07a	0.21 ± 0.07a	0.16 ± 0.07a	0.10 ± 0.07a	0.25 ± 0.07a	0.38 ± 0.08a	0.27 ± 0.08a	0.25 ± 0.08a	0.30 ± 0.07a	0.30 ± 0.07a	0.24 ± 0.07a	0.29 ± 0.07a
	OR	0.24 ± 0.07abc	0.39 ± 0.07ab	0.44 ± 0.07a	0.41 ± 0.07ab	0.43 ± 0.07ab	0.21 ± 0.07abc	0.10 ± 0.07c	0.03 ± 0.07c	0.16 ± 0.07abc	0.13 ± 0.07bc	0.16 ± 0.08abc	0.13 ± 0.08bc	0.26 ± 0.08abc

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accumulation of PP13 in RR was maximal in March and it was not detected in OR (not shown). After bud burst (May), PP26 was less abundant and PP13 disappeared. During June and July, PP13 and PP26 were absent whereas higher molecular weight polypeptides were more abundant (PP87–PP119) or present (PP158). PP26 started to accumulate from August to October in both RR and OR.

Seasonal variations in NSC and NSNC in beech

Concerning NSC, no difference was observed between RR and OR and the concentrations were rather stable through the year (Figure 2c). Starch concentration was significantly lower between December and April compared with October 2007. Between May and June, starch concentration increased and then remained stable until October 2008 with values comparable to those observed in October 2007. Among soluble sugars, sucrose concentration was significantly lower in June and July compared with the other dates. Glucose and fructose concentrations were lower in March and April than during the winter period and were not detected until the end of the growing season, while mannose was not detected at all (Table 4). Amino acid and soluble protein concentrations did not display significant seasonal variation. However, soluble protein composition showed annual variation (Figure 2b). A maximum of seven polypeptides were detected with a molecular weight ranging from 13 to 84 kDa. In RR, a major polypeptide of 26 kDa (PP26) accumulated during the winter period. A polypeptide of 13 kDa (PP13) was also detected in February in RR but the band intensity was lower than that of PP26. The accumulation of PP26 was maximal in February and accounted for 33% of total soluble proteins in RR and for 24% in OR. In February, PP13 accounted for 15% of total soluble proteins in RR and was not detected in OR. During spring growth, PP26 levels decreased (between May and June) to disappear afterward. After the end of stem growth (August), PP26 started to accumulate in both RR and OR.

Discussion

In the stem of mature oak, the outermost sapwood, which is known to contain most of the living parenchyma cells (Kramer and Kozlowski 1979) accumulated more NSNC than the inner sapwood. Such distribution can be advantageous by reducing the distance for N transport to sink tissues (Staswick 1994). By contrast, NSNC were equally distributed between the inner and the outermost sapwood in beech. These contrasting results for the two species are quite similar to those for NSC observed by Barbaroux and Bréda (2002) and in the present study. Thus, we can hypothesize that oak and beech trees have contrasting seasonal dynamics for both C and N pools.

During the dormant period (October 2007–March 2008), deciduous trees are leafless and the stem depends exclusively on stored reserves. In temperate climates, this period is characterized by a decrease in day length and in air temperature. As a consequence, trees undergo endodormancy, which prevents growth during transitory periods of warm temperatures and sustains synchronized spring growth (Rowland and Arora 1997). During this period, C is stored as starch and soluble sugars (Kozlowski and Pallardy 2002) and N is stored as amino acids and proteins (Dickson 1989). In our results, free amino acid concentrations were nearly two times higher than soluble protein concentrations in the sapwood of oak. However, taking into account mean N content of proteins (0.23 g N g^{-1} proteins, Yeoh and Wee 1994) and of amino acids (0.13 g N g^{-1} amino acids), N concentration in the two compounds was thus comparable.

In temperate climates, air temperature often decreases below zero during the endodormancy phase, which can induce irreversible damage at the cellular level species in not cold acclimated (Repo et al. 2008). Consequently, temperate trees have to develop cold hardiness mechanisms to avoid intracellular freezing. Among NSC, we observed starch conversion to soluble sugars, which is a common response to cold hardiness in trees (Kramer and Kozlowski 1979, Piispanen and Saranpää 2001, Poirier et al. 2010) and more particularly in oak species (Thomas et al. 2004, Morin et al. 2007, Repo et al. 2008). Overall NSC concentrations decreased in winter, in the outermost sapwood of oak (Figure 1c), reflecting presumably C use in maintenance respiration (Ögren 2000). Starch to sugar conversion occurred concomitantly with changes in soluble protein composition. During the same period, two polypeptides of 13 kDa (PP13) and 26 kDa (PP26) accumulated in the stem sapwood of both species. Since the stem was considered to be self-sufficient during this period, the polypeptides could originate mainly from existing N pools (soluble proteins, amino acids). Given that amino acid and soluble protein concentrations did not change during the cold period, we hypothesized that the accumulation of PP13 and PP26 resulted from the breakdown of higher molecular weight polypeptides that were less abundant during this period. As PP13 and PP26 accumulated mostly during the period of lowest air temperature, they may have a protective function in cold hardiness. Freezing conditions may induce extracellular ice formation by drawing water from cells and the resulting cellular dehydration may cause damage to membranes and protein denaturation. In overwintering plants, various biochemical changes can take place to cope with such stresses. These include synthesis of antifreeze proteins, which prevent ice formation (Griffith and Yaish 2004), and of soluble sugars, which can preserve proteins and membranes against freeze drying (Hoekstra et al. 2001). As cold acclimation and dormancy development occur at the same time in woody perennials, it is difficult to ensure that qualitative protein changes are specifically related only to freezing tolerance (Pagter et al. 2008). For example, in peach bark, Wisniewski

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Table 4. Monthly NSNC (soluble proteins and free amino acids) and NSC (starch, sucrose, glucose, fructose and mannose) concentrations (% DM) in the most recent rings (RR) and oldest rings (OR) in the stem sapwood of 50-year-old *F. sylvatica* L. (Matt.) Liebl. Most of the glucose and mannose concentrations in October 2007 and from May to October 2008 were zero and these dates were discarded from analysis. Different letters in the same line indicate significant differences between dates (at $P < 0.05$). Asterisks indicate significant differences between compartments: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Compound (% DM)	Compart-ment	October 2007	November 2007	December 2007	January 2008	February 2008	March 2008	April 2008	May 2008	June 2008	July 2008	August 2008	September 2008	October 2008
Soluble proteins	RR	0.12 ± 0.03ab	0.10 ± 0.03ab	0.09 ± 0.03ab	0.10 ± 0.03ab	0.10 ± 0.03ab	0.10 ± 0.03ab	0.08 ± 0.03abc	0.03 ± 0.03c	0.09 ± 0.03abc	0.08 ± 0.03abc	0.08 ± 0.03bc	0.07 ± 0.03bc	0.14 ± 0.03a
	OR	0.06 ± 0.01a	0.03 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.02a	0.06 ± 0.01a	0.04 ± 0.01a	0.05 ± 0.01a	0.07 ± 0.01a
Amino acids	RR	0.10 ± 0.02a	0.09 ± 0.01a	0.08 ± 0.01a	0.11 ± 0.02a	0.12 ± 0.02a	0.10 ± 0.01a	0.13 ± 0.03a	0.05 ± 0.01b	0.11 ± 0.06ab	0.22 ± 0.17ab	0.16 ± 0.05ab	0.08 ± 0.01a	0.12 ± 0.02a
	OR	0.14 ± 0.03a	0.16 ± 0.04ab	0.08 ± 0.02a	0.15 ± 0.03ab	0.09 ± 0.02a	0.10 ± 0.02a	0.15 ± 0.03a	0.09 ± 0.02a	0.08 ± 0.02b	0.08 ± 0.02b	0.08 ± 0.02b	0.09 ± 0.02a	0.10 ± 0.02a
Starch	RR	1.20 ± 0.19ab	0.60 ± 0.09bc	0.34 ± 0.05cd	0.08 ± 0.01e	0.10 ± 0.01e	0.24 ± 0.03d	0.42 ± 0.06cd	1.01 ± 0.15ab	1.42 ± 0.26a	1.09 ± 0.24ab	1.12 ± 0.17ab	1.37 ± 0.22a	1.60 ± 0.26a
	OR	1.29 ± 0.24a	0.68 ± 0.16bc	0.50 ± 0.15cd	0.43 ± 0.14d	0.40 ± 0.14d	0.68 ± 0.16abc	0.90 ± 0.18ab	1.04 ± 0.20ab	1.06 ± 0.21ab	1.19 ± 0.22ab	0.99 ± 0.19ab	1.03 ± 0.20ab	1.03 ± 0.20ab
Sucrose	RR	0.90 ± 0.08ab	1.10 ± 0.08a	0.85 ± 0.08ab	0.90 ± 0.09ab	0.82 ± 0.08ab	0.93 ± 0.08ab	0.87 ± 0.08ab	0.65 ± 0.08bc	0.42 ± 0.09c	0.28 ± 0.12c	0.86 ± 0.08ab	1.01 ± 0.08a	0.93 ± 0.08ab
	OR	0.90 ± 0.08abc	1.00 ± 0.08ab	0.85 ± 0.08abc	0.87 ± 0.09abc	0.95 ± 0.08ab	1.03 ± 0.09ab	0.96 ± 0.08ab	0.49 ± 0.08d	0.57 ± 0.09cd	0.66 ± 0.08bcd	0.72 ± 0.08abcd	0.72 ± 0.08abcd	1.04 ± 0.08a
Glucose	RR	–	0.32 ± 0.04bc	0.48 ± 0.04a	0.49 ± 0.04a	0.43 ± 0.04ab	0.29 ± 0.04c	0.22 ± 0.04c	–	–	–	–	–	–
	OR	–	0.33 ± 0.04b	0.46 ± 0.04a	0.44 ± 0.04a	0.36 ± 0.04ab	0.25 ± 0.04bc	0.19 ± 0.04c	–	–	–	–	–	–
Fructose	RR	–	0.17 ± 0.05bcd	0.32 ± 0.05abc	0.36 ± 0.05a	0.33 ± 0.05ab	0.16 ± 0.05cd	0.05 ± 0.05d	–	–	–	–	–	–
	OR	–	0.07 ± 0.05bc	0.36 ± 0.05a	0.28 ± 0.05a	0.26 ± 0.05ab	0.08 ± 0.05bc	0.05 ± 0.05c	–	–	–	–	–	–

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et al. (1999) characterized a 60 kDa VSP as a dehydrin, which had a role in cold acclimation. The pattern of PP13 and PP26 accumulation fits that described for storage proteins, being present in large quantities in winter and nearly or completely absent during summer (O'Kennedy and Titus 1979, Staswick, 1994). However, the seasonal stability of the pool of total soluble proteins could suggest a protective function rather than a storage function in the stem sapwood.

At the beginning of the growth period (April 2008), bud burst and leaf expansion were initiated allowing the recovery of sap flow. Unlike beech, starch remobilization occurred in the stem sapwood of oak, as already demonstrated by Barbaroux and Bréda (2002). This remobilization could be linked to the high demand of NSC needed during spring reactivation (bud burst, twig growth, early wood growth in the stem). Large multiseriate rays in oak are especially appropriate structures to mobilize NSC from old sapwood rings. However, in June, starch pools did not completely disappear in the stem sapwood of oak, as shown previously for different temperate tree species (Hoch et al. 2003). NSC accumulation can thus be higher than C needs for growth and leads to C sequestration rather than storage (Millard and Grelet 2010). Spring growth of beech seemed to be less dependent on C stored within the stem sapwood, which confirms the results of Barbaroux and Bréda (2002) obtained on younger trees originating from the same site. In fact, the small vessels of beech are less vulnerable to winter embolism (Gasson 1987, Hacke and Sauter 1996) and hydraulic conductivity is partially conserved from year to year. Stem radial growth starts after leaf expansion and C needs are more likely to be supplied by new assimilates rather than from the storage pool within the stem sapwood (Kozlowski 1992, Barbaroux and Bréda 2002). Hoch et al. (2003) suggested that in beech, branches rather than stem contributed more efficiently to new leaf growth. Barbaroux et al. (2003) also showed a significant decrease in NSC levels of beech branches between October (maximum NSC levels) and June (after full leaf expansion). These contrasted results for NSC in oak and beech are not similar to those of NSNC. According to Millard (1988), N is considered to be stored if it could be remobilized from one tissue for the growth or maintenance of another. The seasonal stability of the NSNC pool during the growth period suggests that NSNC are not remobilized from the stem sapwood of either species. The breakdown of PP13 and PP26 could lead to the release of amino acids which could be used to sustain internally wood growth rather than exported to other N sinks. Polypeptides with higher molecular weight were expressed in June in the protein electrophoretic profile of RR, mainly in oak, and could be related to wood growth processes. In poplar stem, xylem-linked proteins were expressed during summer (Vander Mijnsbrugge et al. 2000) at the expense of bark storage proteins.

During the vegetative period (July–October 2008), starch refilling started between July and August in both species whereas stem growth was still ongoing as previously shown (Lacointe et al. 1993, Barbaroux and Bréda 2002). Starch accumulated until the end of the vegetative season in October when NSC reached their maximal concentrations in both species, whereas PP13 and PP26 started to accumulate only with leaf senescence. As N is essential for photosynthesis processes within leaves, the replenishment of N reserves in perennial organs of deciduous tree species starts with leaf yellowing in early fall (Dickson 1989, Sauter and Witt 1997). In fact, 40–80% of foliar N is recycled from senescent leaves in autumn (Chapin and Kedrowski 1983, Millard and Proe 1991, Vizoso et al. 2008), resulting mainly from Rubisco degradation (90%), which constitutes the main source of N reserves (Kang and Titus 1980).

In conclusion, we showed that NSNC pools in both oak and beech did not exhibit contrasting seasonal dynamics as shown for NSC pools. The seasonal course of NSNC is comparable between the two species and was mainly illustrated by marked changes in soluble protein composition in the stem sapwood. These results suggest local changes in protein composition and a function in the internal N cycling within the stem rather than a translocation toward the other parts of the tree. The identity and function(s) of the observed polypeptides require further studies. The stem sapwood is quantitatively the most important storage organ at the tree level in adult trees; however, additional research is needed to investigate the seasonal variation of NSC and NSNC in the other perennial organs (e.g., coarse roots and branches) and to assess their role in C and N balances at the tree level.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Research paper

Nitrogen sources for current-year shoot growth in 50-year-old sessile oak trees: an in situ ^{15}N labeling approach

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We used long-term in situ ^{15}N labeling of the soil to investigate the contribution of the two main nitrogen (N) sources (N uptake versus N reserves) to sun shoot growth from bud burst to full leaf expansion in 50-year-old sessile oaks. Recovery of ^{15}N by growing compartments (leaves, twigs and buds) and presence of ^{15}N in phloem sap were checked weekly. During the first 2 weeks following bud burst, remobilized N contributed ~90% of total N in growing leaves and twigs. Nitrogen uptake from the soil started concomitantly with N remobilization but contributed only slightly to bud burst. However, the fraction of total N due to N uptake increased markedly once bud burst had occurred, reaching 27% in fully expanded leaves and 18% in developed twigs. In phloem sap, the ^{15}N label appeared a few days after the beginning of labeling and increased until the end of bud burst, and then decreased at full leaf expansion in June. Of all the shoot compartments, leaves attracted most of the absorbed N, which accounted for 68% of new N in shoots, whereas twigs and new buds accounted for only 28 and 3%, respectively. New N allocated to leaves increased from unfolding to full expansion as total N concentration in the leaves decreased. Our results underline the crucial role played by stored N in rapid leaf growth and in the sustained growth of oak trees. Any factors that reduce N storage in autumn may therefore impair spring shoot growth.

Keywords: nitrogen partitioning, nutrient uptake, *Quercus petraea*; remobilization, shoot development, spring growth.

Introduction

Among mineral nutrients, nitrogen (N) is a growth-limiting factor for trees in natural forest ecosystems with low anthropogenic N input (Rennenberg et al. 1998, 2009). In floodplain forests characterized by an open ecosystem N cycle, trees are often exposed to a through flow of nitrate (NO_3), which is the predominant N source (Rennenberg et al. 2010). By contrast, in forest ecosystems that develop on soils with limited N availability, reduced N compounds such as amino acids and ammonium (NH_4) are the dominant N sources for trees (Rennenberg et al. 2009). For deciduous broadleaved species growing under these conditions, e.g., European beech (*Fagus sylvatica* L.) and oaks (*Quercus* spp.), growth is seasonal (Rennenberg et al. 2010) and new leaves develop over a few weeks in spring or in a few short-term flushes that occur throughout the growing season (Kozlowski 1971).

Seasonal N cycling is a requisite component of the perennial lifestyle (Staswick 1994, Stepien et al. 1994). In deciduous trees, N is withdrawn from senescing leaves in autumn, and then stored during winter in the form of amino acids and vegetative storage proteins in the bark and wood parenchyma of perennial organs such as roots and stem (Stepien et al. 1994). In early spring, the internal cycling of stored N allows deciduous trees to sustain growth (Millard 1996), particularly when the environmental conditions for N uptake from the soil are not optimal (Murray et al. 1989, Cooke and Weih 2005).

Contrary to carbon (C) remobilization that is sink driven, N remobilization in spring is source driven (Millard and Grelet

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2010) and is not affected by N availability in the soil during the growing season (Millard 1996). Leaf growth is the strongest sink for this N remobilization during spring, with remobilized N reaching up to 90% of the total N used for leaf growth (Millard 1996, Neilsen et al. 1997). Most of this remobilization usually occurs before there is much root uptake (Millard and Proe 1991). The relative contribution of remobilized N and root-supplied N to leaf growth is dependent on soil fertility (Millard 1996) and on tree age (Miller and Miller 1987). Soil N availability during the previous years affects the amount of internally stored N and consequently the amount of N remobilized for spring growth (Frak et al. 2005). Moreover, tree organ biomass increases substantially with age (Bernardo et al. 1997), thus making the N content of perennial organs, and subsequently their storage capacity, increase as they get older (Harrison et al. 2000). This is especially true at the branch and stem level. In both adult beech and oak species, it has been demonstrated that the stem is the major storage compartment for proteins (Valenzuela et al. 2010, 2011).

To date, most studies investigating N sources used for leaf and shoot growth in trees have been carried out on saplings growing under controlled conditions in sand culture pots (Millard and Grelet 2010). To our knowledge, few studies have investigated N sources for spring growth in field-grown trees, but see Millard (1994) for 6-year-old spruce, 8-year-old maple and 10-year-old cherry (Millard et al. 2006).

In spring, a high C remobilization has been shown to occur in adult sessile oak trees [*Quercus petraea* (Matt.) Liebl.], a deciduous porous ring species common in north-eastern France (Barbaroux and Bréda 2002, Barbaroux et al. 2003). This spring C remobilization is accompanied by changes in total soluble protein distribution among tree compartments (Valenzuela et al. 2011). However, Valenzuela et al. (2011) pointed out that it was not possible to determine if these changes in N used for spring growth resulted from current uptake or reserve remobilization. Using ^{15}N labeling to distinguish N uptake from N remobilization (Millard 1996) could help fill the gap in knowledge, but such labeling methods have so far been mostly restricted to young trees due to the difficulty of applying ^{15}N labeling methods at the forest scale (Millard and Grelet 2010).

The aim of the present study was to identify, in the forest, the source of N used for the growth of current-year shoots in 50-year-old sessile oak trees. Our experimental approach was original and challenging, and involved long-term ^{15}N labeling of the NO_3 and NH_4 pools in soil around three trees from bud burst to full leaf expansion. The recovery of labeling in leaves, twigs, buds and phloem exudates was analyzed weekly during shoot growth in order to distinguish N uptake from remobilized N. The results were then compared with current knowledge of the internal N cycle in forest trees.

Materials and methods

Study site and soil description

The study was conducted in a pure stand of naturally regenerated sessile oak [*Q. petraea* (Matt.) Liebl.] in the state forest of Champenoux, France (48°44′ N, 6°14′ E, elevation 237 m). Mean annual temperature was 9.2 °C and mean annual precipitation was 744 mm. In 2009, dominant oak trees were 20 m high and 50–55 years old. Stand density stood at 2553 stems ha^{-1} and basal area was 26.7 m^2 ha^{-1}. The maximum stand leaf area index (LAI) was 6.1 in 2009 as estimated by litter collection during autumn. Stand LAI progression during spring was derived from the canopy interception of global radiation based on above- and below-canopy measurements following Bréda (2003). Radiation was measured every 10 s, and 30-min averages were calculated and stored in data loggers (Campbell Scientific, Courtaboeuf, France). Below-canopy global radiation was measured using a 1-m-long tubular solarimeter (Delta-T device, Cambridge, UK) placed 1 m above the soil level. Incident global radiation was measured with a pyranometer (CMP6, Kipp and Zonen, The Netherlands) at an INRA-monitored weather station near the study site.

The oak stand was located on a cambisol (WRB 2006) lying on deep loam, as described in Bréda et al. (1995). The upper horizon, consisting of a silty clay loam, presented a crumb structure. Hydromorphic spots appeared below 30 cm. Just below this upper compartment, a clay-enriched horizon (Bt) with a clay content of ~45% lay between 60 and 80 cm depth. Below 1 m, clay content gradually decreased while bulk density increased to 1.55 at 1.15 m depth. Site fertility was high; the humus type was oligo-mull, and N content in the upper organic layer (horizon A1) was 2.14 g kg^{-1} (C/N of 13.5).

^{15}N labeling design

Three dominant oaks, designated as N1, N2 and N3, with stem circumferences at breast height of 724, 754 and 898 mm, respectively, were selected for the labeling experiment. The three oaks were situated in a separate 84-m^2 (14 m × 6 m) plot inside the study site. The sparse ground vegetation (hornbeam sprouts, *Anemone nemorosa*, etc.) was removed from the plot in November 2008 (i.e., after leaf fall) and again at the end of March 2009. Since plant litter may retain a significant amount of the label (May et al. 1996), all litter was removed from the soil surface and the plot was carefully cleared just before labeling. The cleaned plot was then divided into 1 × 1 m squares and covered with black plastic sheets to prevent vegetation regrowth and to avoid leaching of the tracer in case of rain (Figure 1). Finally, the plot was enclosed in a wire fence to prevent any disturbance.

The ^{15}N tracer was applied weekly from 10 April (day of the year (DOY) 100) before bud burst to 7 May (DOY 166; see Figure 2) when bud burst was completed. On the day of application, 10 g of $^{15}NH_4{}^{15}NO_3$ enriched to 98 atom% ^{15}N

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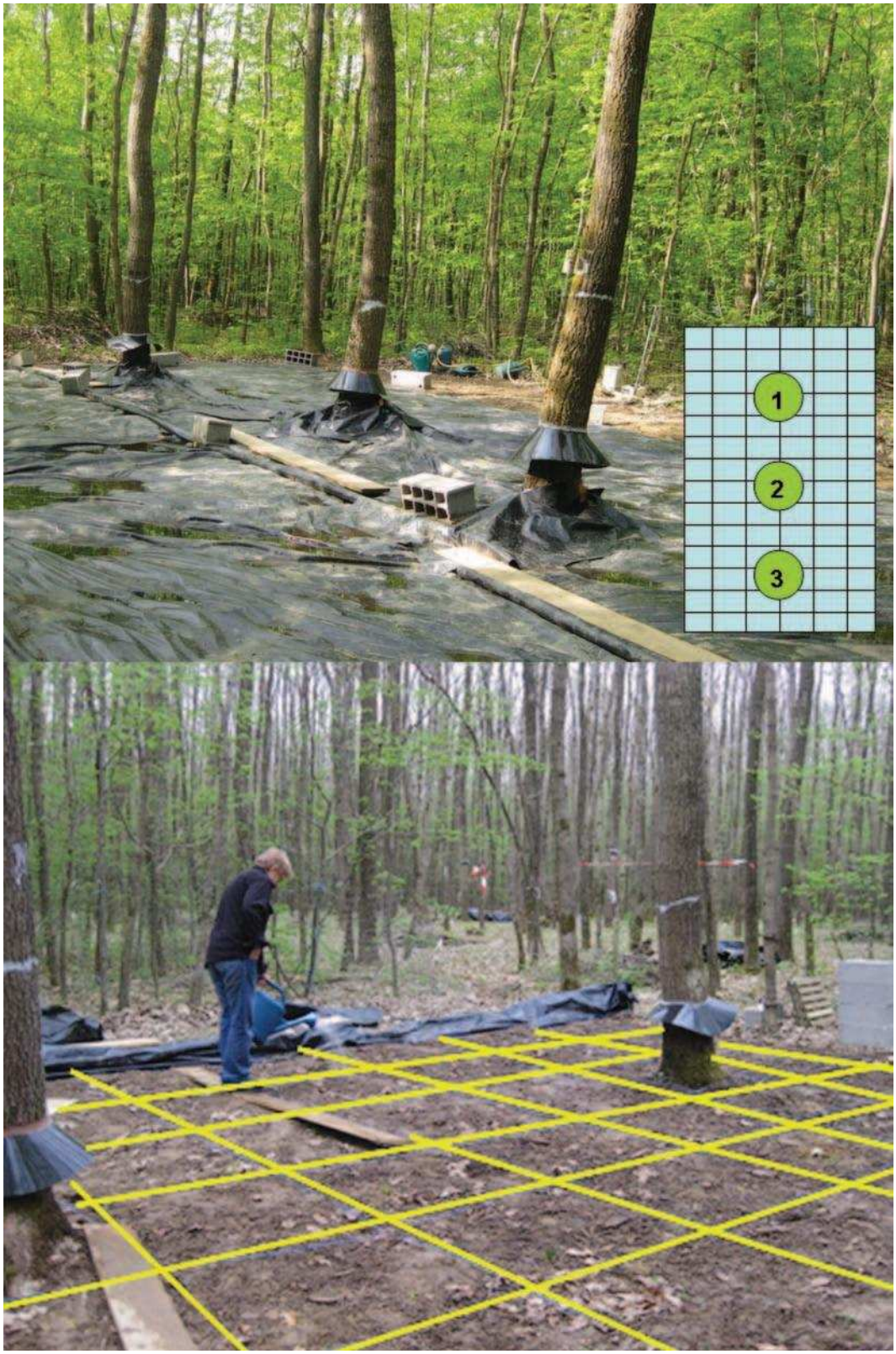

Figure 1. Experimental 15N labeling design in the Champenoux forest (France) showing the three dominant oak trees situated in an 84 m^2 (14 m × 6 m) plot within the study site. The plot was divided into 1-m squares and covered with two superimposed black plastic sheets to prevent leaching and growth of new vegetation.

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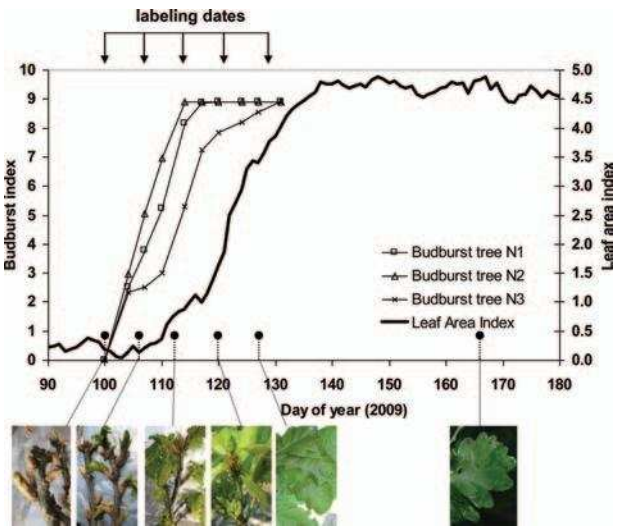

Figure 2. Oak phenology during spring 2009. Bud burst progression as visually assessed for the three oaks (N1, N2 and N3) and stand leaf area index dynamics as derived from global radiation interception. The arrows indicate the labeling dates. Black circles indicate the sampling dates.

(Cambridge Isotope Laboratories, Inc., Andover, MA, USA) were carefully dissolved in 1000 l of freshly deionized water and then applied to the soil around the trees (Figure 1). Each square meter was uniformly watered with 10 l of the ^{15}N solution (Figure 1). Thus, 0.12 g m^{-2} of $^{15}NH_4{}^{15}NO_3$ (i.e., 0.044 g^{15}N m^{-2}) was provided weekly. After each application, the soil surface was covered back with the plastic sheets.

Bud burst monitoring

Bud burst of the studied trees was monitored with binoculars every 2–3 days from the beginning of April to mid-May 2009. Bud development was described according to a six-stage scale (dormant winter buds, swollen buds, broken buds, newly unfolded leaves, completely unfolded leaves and developed leaves with shoot elongation). Bud burst was completed when the tree reached the unfolded leaf stage (Bréda and Granier 1996); this stage corresponds to the onset of both leaf expansion and an increase in LAI.

Soil sampling, water content and N analyses

Before any application of the tracer, three soil samples were randomly collected with an auger around each tree to determine the ^{15}N natural abundance in the soil. Then, before each ^{15}N tracer application, three more soil samples from randomly selected points around each tree were collected. Soil samples were separated into five depth categories corresponding to 10-cm intervals from the silty horizons down to 50 cm in depth where the accumulated clay horizon started. The samples from different depths were collected in separate boxes and stored at 4 °C until the water content was determined and the nitrate and ammonium were extracted. Soil samples were homogenized by sieving them through a 4-mm mesh. In the laboratory, gravimetric soil water content was determined on 40-g sub-samples of soil that had been dried for 48 h at 105 °C. Mineral N (N-NH_4^+ and N-NO_3^-) was extracted after shaking 40 g of fresh soil with 200 mL of 2 M KCl for 1 h. N-NH_4^+ and N-NO_3^- concentrations were determined in filtered extracts using a continuous flow colorimeter (Skalar SA 5000, Breda, The Netherlands). Abundance of $^{15}NH_4^+$ was analyzed by the micro-diffusion technique (Kelley et al. 1991, Sorensen and Steen Jensen 1991). Magnesium oxide (Fluka, ref. 416415/1, Sigma-Aldrich Chemie S.a.r.l., Saint-Quentin Fallavier, France) was added to the solution to recover NH_4^+ on a fiber-glass filter paper soaked with 2.5 M $KHSO_4$. After 1 week, the filters were removed, dried in a desiccator and analyzed. N-NO_3^- was only found at low concentrations in the soil extracts and this prevented us from directly evaluating its ^{15}N signal by mass spectrometry.

Plant material

To collect samples, two sun shoots were shot from the crown of each tree. To determine natural ^{15}N abundance, the first shoots were collected on 10 April (DOY 100), just before bud burst began and before the first ^{15}N application. Thereafter, six shoots (two per tree) were sampled before each weekly labeling application until the end of leaf expansion (mid-June, DOY 166). At each sampling date, the six shoots were separated into leaves, buds (2008 or 2009) and twigs (2008 or 2009) and then frozen in liquid nitrogen in the field. Frozen plant samples were then taken back to the laboratory and stored at –80 °C (–86 °C Freezer® Forma Scientific®, Marietta, OH, USA) before freeze-drying (Dura-Top®, Dura-Dry® FTS Systems®, Stone Ridge, NY, USA). Freeze-dried leaves, buds and twigs were weighed and ground to a fine powder with a laboratory mill (CEPI SODEMI® CB2200, Cergy, France) and then stored in the dark in airtight vials until isotopic analyses.

Collection of phloem sap

Two small disks of bark (12 mm in diameter) were collected from each oak tree at 1.3 m height with a corer (Gessler et al. 2004). The first samples were collected prior to labeling to determine their ^{15}N natural abundance. The inner bark tissue was placed in a 10 ml vial containing 2 ml of ultrapure water and then left to incubate for 5 h at ambient temperature, as described by Rennenberg et al. (1996). Next, the phloem extract solution was filtered in a nylon cartridge (Fisherbrand, 0.2 µm Nyl., diameter 25 mm, ref. W5173P) and vacuum evaporated for 4 h (Maxi-Dry Plus, Heto-model DW1, 0-110, Heto-Holten A/S, Allerod, Denmark). The dried extracts were weighed and then diluted with ultrapure water. A 60-µl aliquot of the resulting solution (containing from about 0.8 to 1 mg of dried phloem extract) was transferred into tin capsules (Elemental Microanalysis, Okehampton, UK, 6 × 4 mm, ref. D1006, BN/139877), freeze-dried (Lyophilizer Christ, Alpha 1-2 LD plus, Osterode, Germany), weighed and analyzed by mass spectrometry.

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Isotopic and elementary analyses

Total N concentration and ^{15}N isotopic abundance in the dry matter of shoot compartments (2–3 mg), phloem sap (0.8–1 mg) and fiber-glass filters (≈50 µg) were measured with an elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled to a Delta-S isotopic ratio mass spectrometer (Finnigan–Mat, Thermoquest Corp., San Jose, CA, USA). Analyses were carried out at our Plateforme Technique d'Ecologie Fonctionnelle (OC 081, INRA, Champenoux, France).

Isotopic calculations

The isotopic abundance for N in atom% (A_N %) is defined as

$$A_N\% = \frac{^{15}N}{^{14}N + {}^{15}N} \times 100$$

The relative specific allocation of new N (RSA_N), which describes the fraction of newly incorporated N relative to the total N in a given compartment (Deléens et al. 1994), was calculated as:

$$RSA_N\% = \frac{\left[A_{N,\ \text{labeled compartment}} - A_{N,\ \text{unlabeled compartment}}\right]}{\left[A_{N,\ \text{labeled soil}} - A_{N,\ \text{unlabeled soil}}\right]} \times 100$$

where $A_{N,\ \text{labeled compartment}}$ is the ^{15}N abundance of the labeled plant compartment, $A_{N,\text{unlabeled compartment}}$ is the ^{15}N abundance of the unlabeled plant compartment (0.36266 atom% ± 0.000295), $A_{N,\text{labeled soil}}$ is the ^{15}N abundance of the soil between 0 and 30 cm (1.32 atom% ± 0.11) and $A_{N,\ \text{unlabeled soil}}$ is the ^{15}N abundance of the unlabeled soil (average value of 0.36658 atom% ± 0.000439).

The isotopic signal of the ^{15}N-NH_4 atom% of the soil between 0 and 30 cm, determined by mass spectrometry throughout the experiment, was quite stable (average value of 0.85642 atom% ± 0.065342). The level of nitrate in the soil was too low to be analyzed by mass spectrometry, so the isotopic signal of the ^{15}N-NO_3 atom% of the soil was assessed by calculation. ^{15}N-NO_3 was brought to the soil in absolute amounts equal to ^{15}N-NH_4 (NH_4NO_3 was 98% ^{15}N) but was diluted in N pools that were very different in size (NH_4 = 18.24 mg NH_4 kg^{-1}; NO_3 = 0.26 mg NO_3 kg^{-1}). We therefore estimated that the label in the nitrate pool was at most 34.64 atom% in the NO_3 pool, while it was 0.86 atom% in the NH_4 pool, thus resulting in an $A_{N,\ \text{labeled soil}}$ value of about 1.32 atom%.

The content of newly incorporated N in each shoot compartment (buds, leaves and twigs) was calculated as follows:

$$\text{new N content (mg)} = RSA_N\ \% \times [N] \times DM$$

where [N] is the N concentration and DM is the dry matter of each compartment.

New N content per shoot was then calculated by summing the new N content of each of its compartments (buds, leaves and twigs). Partitioning of new N (P%) was calculated as the proportion of new N in a given compartment (buds, leaves and twigs) relative to the new N in the whole shoot:

$$P\% = \frac{\text{new N content of the compartment}}{\text{new N content of the shoot}} \times 100$$

Statistics

Data were analyzed by the General Linear Model procedure (Statistica 6.1, Statsoft, Tulsa, OK, USA) to test the effect of tree and date on total N, RSA_N and the partitioning of new N within shoot compartments (buds, leaves and twigs).

Results

Water content, N pools and ^{15}N enrichment of the soil

Soil water content (% dry soil) in the upper 50 cm of soil was stable and close to field capacity during the study period: about 37.2% at a depth of 0–10 cm, 31.6% at 10–20 cm, 28.9% at 20–30 cm and 27.8% at 30–50 cm (Table 1).

Ammonium (N-NH_4^+) was the most abundant form of N in the soil whereas only traces of nitrate (N-NO_3^-) were detected. Concentrations of N-NH_4^+ decreased with soil depth and remained quite stable during the experiment. N-NH_4^+ concentrations averaged about 18.24 mg kg^{-1} at 0–10 cm, 10.48 ± 0.74 mg kg^{-1} at 10–20 cm and 7.28 ± 0.60 mg kg^{-1} at 20–30 cm and then dropped to 4.82 ± 0.46 mg kg^{-1} at 30–40 cm and 2.90 ± 0.43 mg kg^{-1} at 40–50 cm of soil depth. N-NO_3 concentrations were about 0.3 mg kg^{-1} at 0–10 cm of soil depth and then became barely detectable (0.03–0.04 mg kg^{-1}) from 10 to 50 cm (Table 1).

Labeling of the NH_4^+ pool, the dominant N form, was detected within 1 week (16 April) from the first ^{15}N tracer application, with an average value of 0.468 ±0.032 atom% between 0 and 30 cm of soil depth. From 22 April to 7 May, the abundance of the NH_4 pool was relatively stable with an average value of 0.856 ± 0.065 atom% between 0 and 30 cm of soil depth.

Table 1. Soil water content and concentrations of N-ammonium (N-NH_4) and N-nitrate (N-NO_3) pools at 10-cm intervals between 0 and 50 cm of soil depth. Values are the average ± SE of 15 replicates.

Soil depth (cm)	Soil water content (% DM)	N-NH_4 (mg kg^{-1} DM)	N-NO_3 (mg kg^{-1} DM)
0–10	37.2 ± 1.0	18.24 ± 1.75	0.26 ± 0.10
10–20	31.6 ± 0.4	10.48 ± 0.74	0.03 ± 0.02
20–30	28.9 ± 0.4	7.28 ± 0.60	0.04 ± 0.03
30–40	27.9 ± 0.4	4.82 ± 0.46	0.04 ± 0.03
40–50	27.7 ± 0.3	2.90 ± 0.43	0.03 ± 0.02

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Bud burst and shoot growth

On 10 April (DOY 100), the buds were still dormant; they started to swell on 14 April (DOY 104) in trees N1 and N2 but remained dormant in tree N3 (Figure 2). Between 22 and 30 April (DOY 112 and 120), the buds broke and unfolded leaves appeared on trees N1 and N2, whereas the buds had only just broken on tree N3. By 7 May (DOY 127), bud burst was completed and the leaves were unfolded in all three oak trees. The leaves and twigs continued to develop and full leaf expansion was achieved in all three trees by 15 June, 63 days from bud burst (DOY 166), as confirmed by the maximum LAI (Figure 2).

Total concentrations and partitioning of N within the new shoots

Before bud burst in all three trees on DOY 106, total N concentrations in dormant buds were higher than in twigs (Figure 3a) and represented 57% of the total N in the shoot (Figure 3b). On DOY 112, N concentrations in unfolded leaves were higher (4.20 ± 0.05% DM) than in the remaining buds (1.95 ± 0.20% DM) and twigs (1.62 ± 0.35% DM) and represented 56% of the total N in the shoot (Figure 3b). On DOY 120, N concentration in leaves was slightly lower than on DOY 112 and N concentration in new twigs averaged 3.11 ± 0.13% DM. On DOY 166, when all the leaves were fully expanded, N concentrations in all shoot compartments combined were significantly lower than in unfolded leaves only (DOY 112). At this date, N concentrations in leaves, new twigs and buds respectively represented 74, 22 and 4% of the total N in the shoot (Figure 3b).

RSA_N and partitioning of new N within new shoots

On DOY 112, newly absorbed N (new N) accounted for <1% of the total N in unfolding leaves, in developing twigs and in remaining buds (Figure 4a). The fraction of new N RSA_N increased to ~6% on DOY 120 in both unfolded leaves and twigs. When bud burst was achieved (DOY 127), new N accounted for 18% of total N in leaves, 14% in new twigs and 10% in buds. On DOY 166, new N accounted for 27% of total N in fully expanded leaves and for 19% in both twigs and 2009 buds (Figure 4a).

Partitioning of new N within the new shoot (Figure 4b) was similar to that of total N (Figure 3b). At the early growth stage (DOY 106), new N was mainly located in dormant buds (62%) and only represented ~ 38% in twigs. From DOY 112 to 166 (full leaf expansion, $P = 0.002$), new N was increasingly allocated to growing leaves (from 58 to 80%) while new N allocated to developing twigs decreased over time (from 32 to 17%, $P = 0.004$, Figure 4 and Table 2).

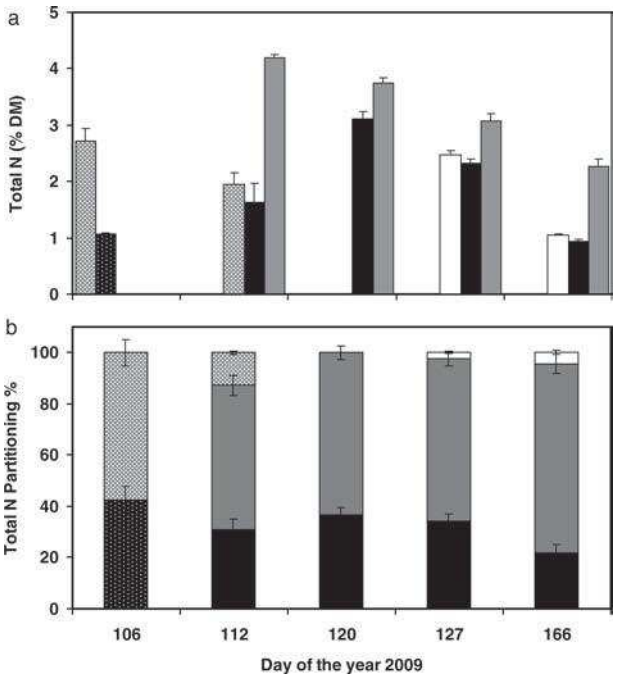

Figure 3. Changes in total N concentrations (a) and partitioning (%) (b) in 2008 (dotted white) and 2009 (white) buds, 2008 (white) and 2009 (dotted white) buds, and leaves (grey) of 50-year-old sessile oak trees from bud burst to completion of leaf expansion. Values are the average ± SE, $n = 6$.

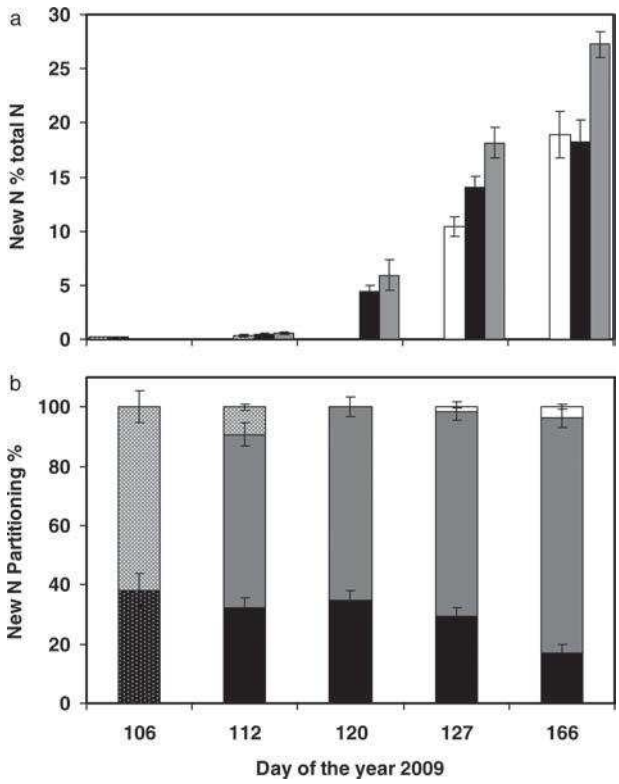

Figure 4. Changes in RSA_N (a) and new N partitioning (b) in 2008 (dotted white) and 2009 (white) buds, 2008 (white) and 2009 (dotted white) buds, and leaves (grey) of 50-year-old sessile oak trees from bud burst to completion of leaf expansion. Values are the average ± SE, $n = 6$.

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Table 2. Analysis of variance in the effects of tree and date on total N, RSA_N and partitioning of new N within buds, leaves and twigs. *P* values and levels of significance are indicated: ns, non-significant; $*P < 0.05$; $**P < 0.01$ and $***P < 0.001$.

	Variable	Total N	RSA_N	Partitioning of new N
Shoot compartments				
Buds 2008	Tree	0.083 ns	0.090 ns	0.809 ns
	Date	0.033*	0.32 ns	0.000***
Buds 2009	Tree	0.99 ns	0.31 ns	0.372 ns
	Date	0.000***	0.004**	0.025*
Leaves	Tree	0.749 ns	0.779 ns	0.264 ns
	Date	0.000***	0.000***	0.002**
Twigs 2009	Tree	0.687 ns	0.734 ns	0.040*
	Date	0.000***	0.000***	0.004**
Phloem sap	Tree	0.260 ns	0.971 ns	–
	Date	0.009**	0.000***	–

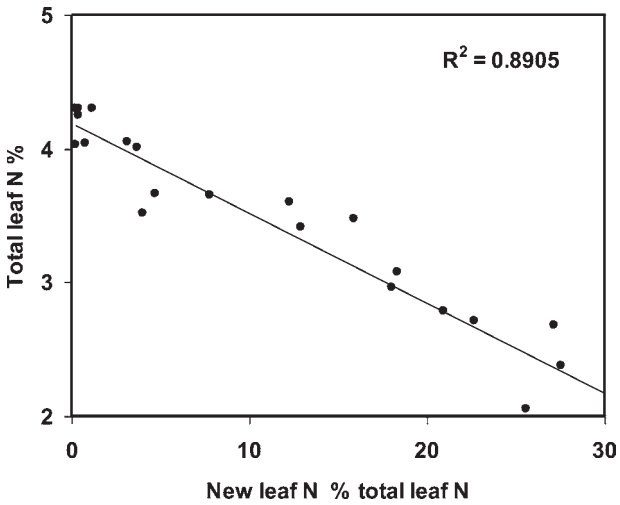

Figure 5. Linear regression between total N concentrations and leaf RSA_N from bud burst to completion of leaf expansion with its associated coefficient of determination (r^2).

Figure 5 illustrates the correlation between total leaf N and the fraction of newly incorporated N (RSA_N, %) in leaves during the sampling period. Total leaf N concentrations were negatively correlated to RSA_N ($r^2 = 0.89$). Total leaf N was at its highest concentration (4.2%) when RSA_N was only about 0.5%, and both evolved inversely during leaf development. At full leaf expansion in June, total N concentrations dropped to 2.27% whereas RSA_N reached 27%. Such a strong correlation was not found for the other compartments.

Total N concentrations and RSA_N in phloem sap

From bud burst to full leaf expansion, total N concentration decreased significantly ($P = 0.009$) in the phloem sap collected at a height of 1.3 m on the stem (Figure 6). New N appeared shortly after the first ^{15}N labeling application. RSA_N was 4 ± 2% on DOY 112, increased to 11 ± 1% on DOY 120, to 17 ± 3% on DOY 127, and then dropped to 9 ± 1% on DOY 166 when the leaves were fully expanded ($P = 0.005$, Figure 6 and Table 2).

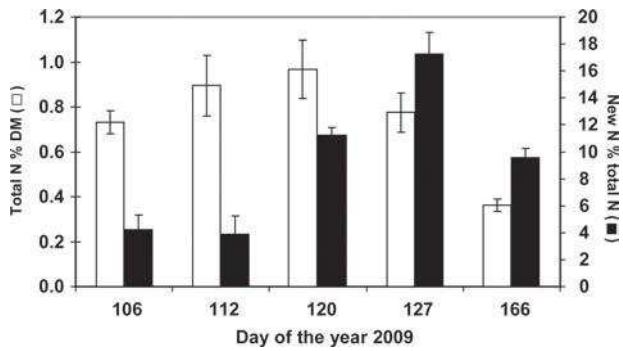

Figure 6. Changes in total N concentration (white) and RSA_N (dark) in phloem sap collected at 1.3 m in height on 50-year-old sessile oak stems from bud burst to completion of leaf expansion. Values are the average ± SE, $n = 6$.

Discussion

Soil N form for root uptake

In temperate forest ecosystems, tree N demand is met mainly by ammonium (NH_4^+) and nitrate (NO_3^-) mostly taken up by mycorrhizal roots from the organic and mineral soil layers (Glass and Siddiqi, 1995). During our experiment, soil organic N was mineralized to NH_4^+ but was not further transformed into NO_3^-. About 22 mg m^{-2} of ^{15}N-NO_3^- was sprinkled weekly on the soil surface while only 25% of applied N-NO_3 (5 mg m^{-2}) was recovered as extractable N throughout the experiment. Such a result led us to hypothesize that applied nitrate was either rapidly taken up by the trees during spring growth or immobilized by microorganisms. Microbes are stronger competitors for applied nitrate than oak roots because they are present in the whole soil volume. If microbes do indeed immobilize and transform applied nitrate, this would considerably reduce the amount of nitrate available for the trees. Even though we did not measure gross N flux, nitrification is apparently rather low in the mineral soil at our site. According to the main soil and site characteristics, ammonium is the dominant mineral N form. Thus, it would not be surprising that NH_4^+ was the predominant source for root uptake by sessile oak trees between April and May. NH_4^+ concentrations decreased with soil depth but varied little during the 5 weeks of ^{15}N tracer applications from April to May (results not shown). Covering with the plastic sheet prevented changes in soil moisture, and moisture content has been recognized as an important driver for N mineralization in forest soils (Andrianarisoa et al. 2009). On the other hand, the plastic sheet may have limited gas exchange between the soil and the atmosphere, and this may have affected soil microbial activity. The slight variations in the ^{15}N atom% of

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NH_4^+ from 22 April (DOY 112) to 7 May (DOY 127) that we observed could be the result of a steady state between mineralization and root uptake. These variations also led us to expect a high rate of turnover in the NH_4^+ pool, with perhaps 80% of the applied label taken up or otherwise utilized (e.g., by other organisms) during those 5 weeks. Within this time frame, the weekly tracer application allowed us to maintain optimal conditions for such an experiment. Nevertheless, we cannot exclude uptake of organic N compounds like amino acids as shown by Gallet-Budynek et al. (2009); however, with respect to the site conditions (soil type, pH and climate), this would probably be a source of negligible N.

Nitrogen content of current-year shoot leaves

Leaves on current-year shoots contained high N concentrations that declined progressively from the beginning of leaf unfolding in April until full expansion in June. The same tendency has been observed for Sitka spruce and sycamore tree leaves (Millard 1994). In the evergreen Japanese oak (*Quercus glauca* Thunb.) and the deciduous Japanese maple (*Acer palmatum* Thunb.), leaf N concentration was high at the beginning of leaf expansion and then decreased to reach a steady state after full leaf expansion (Koyama et al. 2008). In 26-year-old oak (*Quercus serrata* Thunb. ex. Murray), leaf N concentration was the highest in May just after bud burst, then decreased gradually toward early June and tended to remain relatively constant until the beginning of leaf senescence (Migita et al. 2007). The decline in leaf N concentration during leaf expansion essentially results from the dilution of N by increasing C biomass (Chapin 1980). However, this decline could also result from changes in the amount and kind of protein synthesized in leaves over time. A progressive decrease in N incorporation into the ribulose bisphosphate carboxylase-rich fraction has indeed been demonstrated on rice plants through the use of ^{15}N labeling by Mae and Ohira (1982).

Nitrogen sources for current-year shoot growth

In our study, the appearance of ^{15}N in buds, leaves and twigs from bud burst to the completion of shoot growth was used to determine when the uptake of soil N by roots started to contribute to spring growth. In April, N uptake was limited by low soil temperatures, which affected the mineralization rate and root activity, and by the absence of transpiring leaves. Two days from the beginning of bud burst (16 April), the ^{15}N tracer was allocated to swollen buds and twigs (<1%) and continued to increase thereafter, demonstrating that in adult sessile oak, N uptake started early and participated, to a small extent, in providing N for bud burst and shoot growth. This early N uptake from the soil could be related to sessile oak hydraulic properties. As a ring-porous species, sessile oak achieves 30% of annual radial stem growth before leaf expansion in spring (Bréda and Granier 1996). During our experiment, stem growth started on DOY 103, as detected by automatic microdendrometers (data not shown); thus, early wood growth had already started before the first labeling application. This type of phenology is associated with winter embolism of the large xylem vessels (Cochard et al. 1992), and indicates that water flow pathways are restored each spring before the onset of transpiration (Bréda and Granier 1996). This makes early root N uptake from soil possible as soon as a threshold soil temperature is reached. Even once the water flux is restored, notwithstanding root function limitations, nutrient transport through the xylem toward new shoots is not really efficient since new flushing leaves are too small and immature to transpire enough to ensure high water and nutrient movement inside the tree. All these reasons might explain the low contribution of newly absorbed N to N accumulation during oak regrowth.

By contrast, N remobilization from storage organs to growing tissues within the tree crown is not influenced by N availability and uptake from the soil (Millard and Proe 1991, Wendler et al. 1995, Millard 1996, Frak et al. 2005). In our study, remobilized N was the main contributor to spring growth of the shoots on 50-year-old sessile oaks and accounted for ~90% of total N in leaves and twigs at the early growth stages (DOY 120). Previous studies have also found that remobilized N contributed significantly to leaf expansion in young temperate tree species, accounting for >60% of total leaf N in white birch (Wendler and Millard 1996) and sycamore maple (Millard and Proe 1991), for >75% and almost 90% in the lateral and top leaves of Japanese oak (*Q. serrata*) (Ueda et al. 2009), and for 80% of total N in pedunculate oak leaves (Vizoso et al. 2008). Evidently, in mature oak trees, shoot growth is also mostly dependent on N reserves, especially at the first stage of bud burst. The high foliage biomass (~7 kg of dry matter per tree—not shown) in 50-year-old trees implies a high supply of stored N. In fact, storage capacity increases with age due to the increase in sapwood biomass, as demonstrated for C storage (Genet et al. 2010). Therefore, internal cycling of N becomes increasingly more important for the overall N economy of mature trees (Miller 1986).

Nitrogen remobilization can typically occur for 20–30 days before the roots become active for N uptake, e.g., in apple (Neilsen et al. 1997; Guak et al. 2003), poplar (Millard et al. 2006), cherry (Grassi et al. 2002), pear (Tagliavini et al. 1997) and spruce (Millard and Proe 1993). In a few species (e.g., the mountain ash *Sorbus aucuparia*), root uptake of N did not start until N remobilization ended (Millard et al. 2001). However, in other deciduous trees such as walnut (Frak et al. 2002) and silver birch and in evergreen trees such as Scots pine (Millard et al. 2001), concomitant N remobilization and uptake from the soil was observed. In our study, early new growth in mature sessile oak is less decoupled from soil N uptake compared with some other tree species. When bud burst was achieved

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(DOY 127), N assimilated from root uptake accounted for 18% in leaves and 14% in twigs and increased respectively to 27 and 18% when full leaf expansion was reached in June. This is consistent with the results of Millard and Proe (1991) and Grassi et al. (2002), who found that after the end of bud burst, the source of N for leaf growth switches from predominantly internal N remobilization to root uptake.

Nitrogen partitioning within shoot compartments

Newly assimilated N within oak shoots was higher in leaves (68%) than in twigs (28%) from the early stages of spring growth in April until full leaf expansion in June. Leaves were also the major sink for new N during the first 6 weeks of growth in 2-year-old beech trees (Dyckmans and Flessa 2002). In fact, the distribution of N compounds into each organ is strictly controlled by sink activity (Osaki et al. 1995). Leaves are the major organ for N accumulation during the vegetative period (Millard and Thomson, 1989) and their development creates a high sink for N. High N partitioning to leaves is essential for photosynthetic activity (Evans and Seemann 1989) as a large part of leaf N (up to 75%) is present in the chloroplasts, much of it in thylakoid membranes and soluble proteins of the Calvin cycle, in particular the enzyme Rubisco (Evans 1989).

Nitrogen translocation within phloem sap

The N is transported from roots to new shoots in trees through long-distance transport. In such long-distance transport of N, phloem can contribute significantly with xylem to the allocation of N to the new shoots, notably through bidirectional exchanges of N compounds between the phloem and xylem vessels, as demonstrated in several herbaceous and woody species (Pate and Layzell 1981, Gessler et al. 2003). A functional link between xylem conductance and phloem, mediated by the active exchange of ions from phloem to xylem via living parenchyma cells, would explain the magnitude of these exchanges in the hydraulic architecture of trees (Zwieniecki et al., 2004).

In the present work, we did not assess xylem sap changes to limit sampling shots and to prevent harmful crown damage. Moreover, we were not certain that the xylem circulation in oak branches was fully restored at this time of the year. Our results show that the RSA_N in the phloem sap of oak trees increased during bud burst (DOY 106–127), a period characterized by a high N demand for growth, and then decreased when the leaves were fully expanded by mid-June (DOY 166). In fact, the translocation of mineral and/or organic N occurs via the xylem and is largely controlled by the direction of the transpiration stream. During spring tree growth, bidirectional exchanges of N compounds between the phloem and xylem (Gessler et al. 1998, 2003, Weber et al. 1998) could explain the observed increase in RSA_N during bud burst. Such a result tends to reinforce the role of phloem in transporting absorbed N at the early spring stage in adult oak, while xylem is not fully rebuilt. There are few chances that the early appearance of ^{15}N in the stem phloem sap was due to a mineral assimilation of N at the foliar level, at least during the first 3 weeks, because the percentage of new N in the phloem far exceeded the percentage of new N in the leaves during the same period, and expanding leaves are more likely to be sinks for phloem transport rather than sources. So we can reasonably conclude that mineral N assimilation occurred exclusively in the roots for the first few weeks after bud burst.

Later on, at the end of leaf expansion, the new N in phloem sap could also have originated from mineral N assimilated at the foliar level. The extent of NO_3 reduction in roots and shoots in woody plants has been shown to be dependent upon the N form, and if NH_4 is present in the root medium of trees, NO_3 reduction can be suppressed (Thomas and Hilker, 2000). The fact that ammonium ($N\text{-}NH_4^+$) was the most abundant form of N in the soil, whereas only traces of nitrate ($N\text{-}NO_3^-$) were detected, precludes a massive assimilation of nitrate at the foliar level in our study trees. The decrease in RSA_N in the phloem sap that occurred when the leaves were fully expanded (DOY 166) could be partly explained by dilution with non-labeled N since we stopped the labeling on DOY 127. This dilution in the phloem sap could also have been caused by the leaves re-releasing old N, due to a high turnover in Rubisco. This hypothesis is strengthened by the fact that leaf N concentrations decreased as new N concentrations in the total N increased (see Figure 5).

In conclusion, stored N was the major source of N for growth of the current-year shoots on 50-year-old sessile oak trees. Nitrogen uptake by the roots was concomitant with N remobilization and increased only after the end of bud burst. Our findings underline the importance of N reserves for spring growth in adult sessile oak trees. Further studies are required to investigate the relative contribution of storage organs such as roots, stem and branches to the remobilization of N for spring growth. The form of remobilized N (protein or non-protein fraction) primarily allocated to the growing sites also needs to be assessed, as does the contribution of newly assimilated N to N storage for the next season.

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