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## ISOTOPIC APPROACH TO TRACE WATER AND NUTRIENT DYNAMICS IN FOREST SOILS

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### INTRODUCTION

Forest ecosystems are generally adapted to unfavourable environments, but increasingly encounter considerable constraints, particularly due to climate change, and in certain areas increased biomass removal. Forest soil fertility is traditionally estimated by measuring nutrient pools and water stocks in multiple soil horizons and by quantifying input and output fluxes of elements in the ecosystem (Legout *et al.*, same issue). Functioning of forest ecosystems is characterised by strong and variable interactions over time between physical, chemical and biological processes, which makes it difficult to define the concept of fertility. The *in situ* monitoring of an ecosystem provides access to net fluxes, in which soil represents a “black box”. This “box” receives influxes (sources) and emits outfluxes (sinks) that conventional methods do not distinguish or explain.

Many research questions about nutrient cycles remain:

- What is the contribution of atmospheric deposition (wet and dry deposition, direct foliar absorption)?
- How can we better understand mineral weathering and degradation of soil organic matter? Which organisms are involved? What are the sources? What are the fluxes? How much organic matter is stored in the soil in an available form?
- What is the contribution of processes related to trees (spatial and temporal location of root uptake, allocation and immobilisation in the different compartments)?

The role of biogeochemical cycles is still not well-known in the functioning of forest ecosystems, the contribution to soil fertility, their ability to resist certain constraints (natural and anthropogenic) and their capacity to maintain or restore ecosystem quality. Better understanding is needed about water and nutrient dynamics within and between different compartments of the ecosystem. Use of geochemical and/or isotopic tracers is an innovative and complementary approach to more conventional studies, which nonetheless remain necessary for studying biogeochemical cycles. Use of these tools has increased due to the recent development of high-performance analytical techniques and the diversification of tracers.

In environmental studies, these tracers are relevant for observing the dispersion of specific elements or groups of elements through the ecosystem and identifying sources that feed the exchangeable compartment of soil, the water and living organisms. Some are helpful in identifying physico-chemical or biological processes.

After an overview of the methodological approaches used in this field, we present results of recent studies that investigated the functioning of forest ecosystems. These examples illustrate the appeal and usefulness of this approach for improving understanding of water and nutrient bioavailability, soil fertility, and mineral and water nutrition of trees.

## **GENERAL CHARACTERISTICS OF THE METHOD**

### **Definition and principle of the method using isotopic tracers**

Each chemical element has several isotopes with the same number of electrons and protons, but a different number of neutrons in the nucleus. Isotopes of an element have almost identical chemical properties, but their mass and nuclear properties may differ: some are radioactive while others are stable. Radioactive isotopes have been used for a long time to date continental environments, with application in soil studies ( $^{14}\text{C}$ ) to estimate the age of soils and quaternary sediments. Radioactive isotopes, such as  $^{32}\text{P}$  or  $^{33}\text{P}$ , are used in the laboratory to determine potentially available P-reserves in soils. Various stable isotopes are used as tracers of sources and/or processes. Table I presents the stable isotopes of elements used in this study, their abundance and the range of currently known natural isotopic variations.

### **Stable isotopes: tracers of sources and/or processes**

One essential condition for considering an isotopic system a suitable tracer of sources is a significant difference in the chemical and/or isotopic compositions of these sources. Their contribution to a mixture can be quantified in various compartments, and their isotopic composition should not be modified by bio-physicochemical reactions. Strontium (Sr) is widely used in this context to study processes on the Earth's surface, such as soil mineral weathering, soil formation, and the origin and mobility of cations.

Some tracers, such as isotopes of light elements (*e.g.* nitrogen  $^{15}\text{N}$ , carbon  $^{13}\text{C}$ , magnesium  $^{24}\text{Mg}$ , calcium  $^{44}\text{Ca}$ , oxygen  $^{18}\text{O}$ ), undergo isotopic fractionation during physico-chemical or biological reactions. This means that one isotope, due to its heavier or lighter weight, is preferentially involved in a reaction. The isotopic composition of the product of this reaction will differ from that of the initial substrate. These tracers highlight biological processes (*e.g.* tree uptake, plant internal translocations), chemical reactions, interactions between elements, or dynamics of certain elements. In certain contexts, they are useful for tracing element sources.

### **Determining isotopic composition of natural samples**

Analytical methods are not explained in detail here because each isotopic system involves a specific protocol<sup>(1)</sup>. Regardless of the tracer used, accurate measurements are always required to identify small isotopic variations between the soil, water and vegetation compartments (table I, p. 61). The first step is to adequately and representatively sample the compartments<sup>(2)</sup>. For solid matter

(1) For more details, see: [http://www-pub.iaea.org/MTCD/publications/PDF/TCS-32\\_fr\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/TCS-32_fr_web.pdf), pp. 107-136 or <http://stableisotopefacility.ucdavis.edu/>.

(2) For water samples, see: [http://www-pub.iaea.org/MTCD/publications/PDF/TCS-32\\_fr\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/TCS-32_fr_web.pdf), pp. 107-136.

TABLE I

**Isotope abundance, isotopic ratios of elements commonly  
used as tracers in environmental studies and intervals of natural isotopic  
variations currently known in the environment  
( $\delta$  notations are calculated in reference to international tracers specific to each element)**

Element	Light isotope		Heavy isotope		Isotopic composition	Range (‰)
	Symbol	Abundance (%)	Symbol	Abundance (%)		
Carbon	<sup>12</sup> C	98.89	<sup>13</sup> C	1.11	$\delta^{13}\text{C}$	
Nitrogen	<sup>14</sup> N	99.63	<sup>15</sup> N	0.37	$\delta^{15}\text{N}$	
Hydrogen	<sup>1</sup> H	99.98	<sup>2</sup> H	0.01	$\delta^2\text{H}$	
Oxygen	<sup>16</sup> O	99.76	<sup>18</sup> O	0.2	$\delta^{18}\text{O}$	
Magnesium	<sup>24</sup> Mg	78.7	<sup>26</sup> Mg	11.17	$\delta^{26}\text{Mg}$	5
			<sup>25</sup> Mg	10.13	$\delta^{25}\text{Mg}$	
Calcium	<sup>40</sup> Ca	96.97	<sup>44</sup> Ca	2.06	$\delta^{44}\text{Ca}$	5
			<sup>42</sup> Ca	0.64		
			<sup>43</sup> Ca	0.145		
			<sup>46</sup> Ca	$33 \times 10^{-4}$		
			<sup>48</sup> Ca	0.18		
Strontium	<sup>86</sup> Sr	9.86	<sup>87</sup> Sr	7.02	$\delta^{87}\text{Sr}/^{86}\text{Sr}$	
			<sup>85</sup> Sr	0.56		
			<sup>88</sup> Sr	82.56		

(e.g. soil, vegetation), samples must be digested and purified to collect the isotopes in the gas phase (e.g. C isotopes) or liquid phase (e.g. Mg or Ca isotopes). Liquids are treated in specific ion-exchange resins to purify the studied element before measuring its isotopic composition by mass spectrometry (mass spectrometer or ICP MS multi-collection). The principle of the measurement is to separate and detect the elements according to their mass and charge.

Another approach is to provide a known amount of tracer(s) in excess in a compartment, which is called “isotopic enrichment”. The concept is to create a strong gradient in isotopic composition, which facilitates monitoring the kinetics of element transfer through the ecosystem. Tracing can be accomplished *in* or *ex situ* to study the processes finely. Enrichment does not require such accurate analytical isotopic precision, at least in the initial phase, but the dilution related to redistribution in the long-term ecosystem requires sophisticated determinations. The high cost of the tracer often limits its use in experimental systems.

## DYNAMICS OF ELEMENTS IN FOREST ECOSYSTEMS

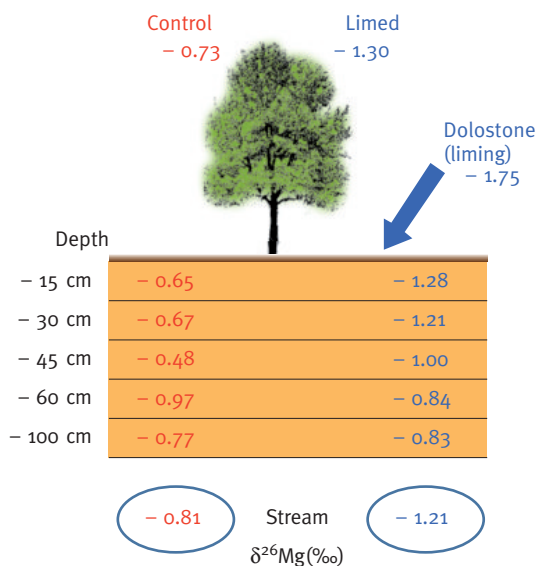
### Acidification of soil and water and possible restoration by liming

In 2003, small forested catchments with acidic soils developed on granite (Cornimont) and sandstone (Senone) bedrock were amended with limestone, dolostone and gypsum in the Vosges

Mountains (France). Since then, soil, water and forest-stand quality, as well as animal communities, have been continually monitored. Figure 1 shows results of the Mg-cycle of the granitic Cornimont catchment. Isotopic variations measured in the control catchment (not amended) were attributed to key processes causing Mg-isotope fractionation (Bolou Bi *et al.*, 2012). The liming material has a negative isotopic signature ( $\delta^{26}\text{Mg}$  of  $-1.75\text{‰}$ ) compared to other compartments and sources of Mg in the system. In the amended catchment, the negative  $\delta^{26}\text{Mg}$  of certain compartments seven years after treatment indicate that the Mg in lime has reached tree leaves, participated in saturation of the soil-exchange complex up to 45 cm deep, and begun to leave the catchment in the streamwater. These results corroborate those from more conventional techniques, which show an increase in Mg concentration in the exchangeable pool of upper soil layers, in tree leaves, and at the outlet of the amended catchment. The isotopic approach demonstrates that the Mg increase after amendment is the dissolution of liming material and not the transfer of pre-existing Mg in the ecosystem.

FIGURE 1

**Mg ISOTOPIC COMPOSITION OBSERVED IN SOIL, WATER AND TREES (BEECH) OF THE CORNIMONT CATCHMENT (VOSGES) SEVEN YEARS AFTER LIMING. THE CONTROL CATCHMENT IS ON THE LEFT AND THE LIMED CATCHMENT IS ON THE RIGHT (BOLOU BI *et al.*, IN PRESS)**



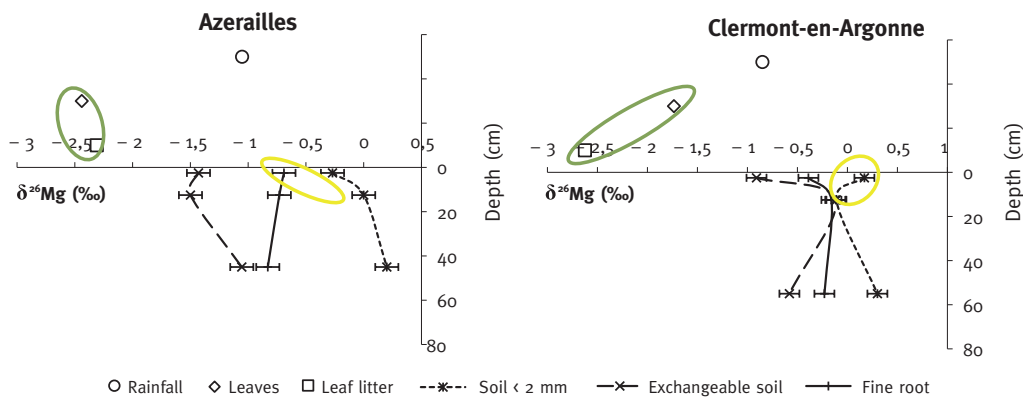
### Geochemical functioning of soils sensitive to compaction

Intensification of forestry leads to increased traffic of heavy machinery. This increases the risk of degradation of compaction-sensitive fine-textured soils, which are abundant on the Lorraine plateau (France). Two experimental sites, Clermont-en-Argonne (Meuse) and Azerailles (Meurthe-et-Moselle), were established in Lorraine (Cooperation INRA/ONF). Soils on both sites have similar physical properties, *i.e.* a 50 cm silty layer resting on a clayey substrate. Both soils are rich in Ca and Mg in

the clayey layer, but the silt layer at Clermont-en-Argonne is more acidic ( $\text{pH} < 4.5$ ) than at Azerailles ( $\text{pH} > 4.5$ ) (Ranger *et al.*, 2012).

Isotopic analyses of ecosystem compartments were performed to study the Ca and Mg cycles and to link soil chemical properties to soil physical properties, soil susceptibility to compaction and the rate of porosity recovery after compaction. At both sites, soils and trees had similar relative Mg isotopic variations, which confirms significant isotope fractionation by biological or physico-chemical processes (figure 2). Isotopic fractionation existed between tree leaves and the forest floor only at Clermont-en-Argonne ( $\delta^{26}\text{Mg}$  of  $-1.7\text{‰}$  and  $-2.6\text{‰}$ , respectively). This suggests strong Mg retranslocation before litter fall, which probably resulted from the low Mg availability in the soil of this site. The multi-isotope approach (Mg, Ca, Sr) assessed functionality of the physical system, its relation to fertility and the potential impact of compaction.

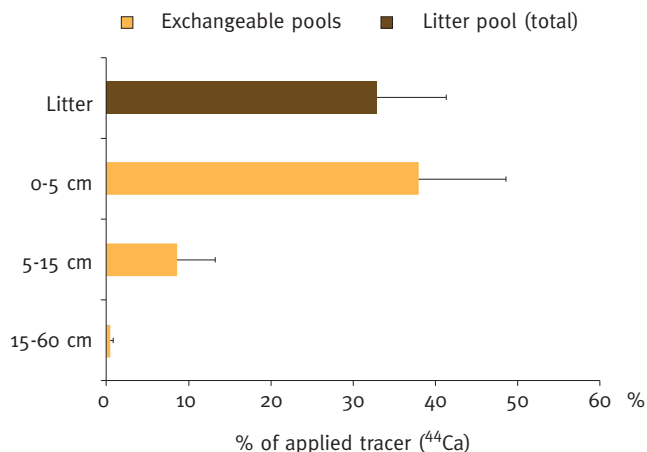
**FIGURE 2 Mg ISOTOPIC COMPOSITION MEASURED IN SOIL (BULK AND EXCHANGEABLE STORES), RAINWATER AND TREES AT THE AZERAILLES AND CLERMONT-EN-ARGONNE EXPERIMENTAL SITES**



### Nutrient cycling in forest ecosystems on acid soil

To better understand Ca and Mg cycles, a multi-isotope tracing ( $^2\text{H}$ ,  $^{26}\text{Mg}$ ,  $^{44}\text{Ca}$ ) experiment was conducted at an experimental site in the Breuil-Chenue forest (Nièvre department, France), in a 30-year-old beech plantation growing on a soil poor in Ca and Mg (Van der Heijden, 2013). Tracers were sprayed in a soluble form on the soil surface in April 2010. Dynamics of these isotopes in ecosystem compartments were then monitored regularly. Retention of Ca (and to a lesser extent Mg) in humus (the OI layer) and mineral soil was high (figure 3, p. 64), confirming the important role of organic exchange. Use of these tracers highlights the slow redistribution of Mg and Ca in the ecosystem from the forest floor to the soil and from the roots to the tree canopy. This behaviour is attributed mostly to the affinity of these ions for organic material. This study also confirmed the conservative nature of Mg and Ca cycles, even under highly acidic conditions, as their loss in drainage after two years was zero. The isotopic balance of the ecosystem two years after tracer enrichment was nearly 100%. This demonstrates the great appeal of the isotope-tracing approach, especially on sites also using the conventional flux-measurement approach, as it provides a more relevant assessment than using only one approach.

**FIGURE 3 PERCENTAGES OF APPLIED TRACER ( $^{44}\text{Ca}$ ) IN THE FOREST FLOOR AND ON THE EXCHANGE COMPLEX IN SOILS AT DIFFERENT DEPTHS, TWO YEARS AFTER ENRICHMENT (BREUIL-CHENUE EXPERIMENT, NIÈVRE, FRANCE)**



## DEPTH OF WATER AND NUTRIENT UPTAKE BY VEGETATION

### Climate change and water reserves in Mediterranean soils

In stands of Aleppo pine in Cyprus, O isotopes were used to understand tree response to drought and drought's relation with tree mortality (Sarris *et al.*, 2013). Oxygen's  $^{18}\text{O}$  isotope is heavier than its  $^{16}\text{O}$  dominant atom, and water that contains more  $^{18}\text{O}$  requires more energy to evaporate. The O isotopic signature ( $\delta^{18}\text{O}$ ) of rainwater is thus positively correlated with temperature. The isotopic signature  $\delta^{18}\text{O}$  measured in tree rings reflects the isotopic composition of the water taken up from the soil by roots during tree-ring formation.

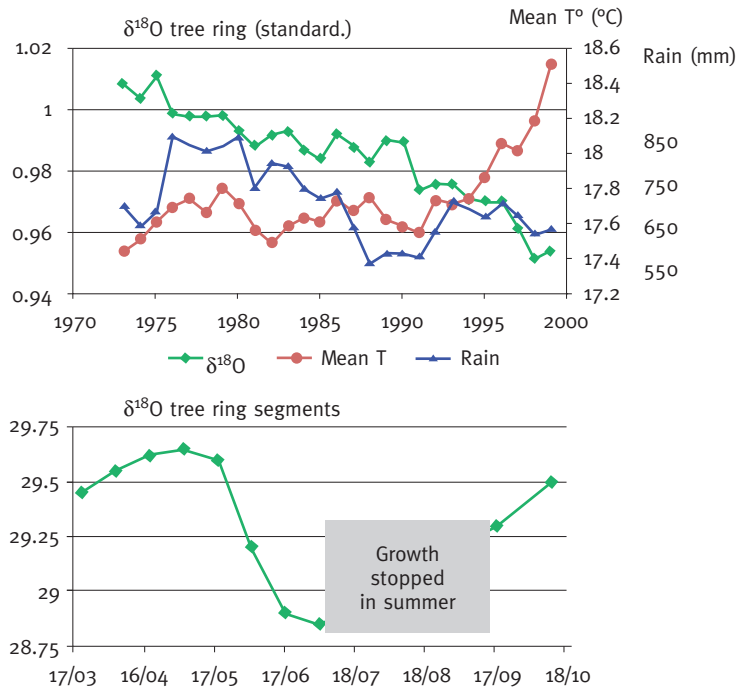
A negative correlation appears between the  $\delta^{18}\text{O}$  of tree rings and mean annual temperatures from 1970-2000 (figure 4a, p. 65). This contradicts the usual relation between  $\delta^{18}\text{O}$  and temperatures, but it parallels the gradual decrease in rainfall over time. In the Mediterranean region, deep water, low in  $^{18}\text{O}$ , comes from winter precipitation. Rainfall in late spring and summer, enriched in  $\delta^{18}\text{O}$ , is quickly used by vegetation and evaporated, never reaching deep soil layers. The observed trend suggests that trees are using water taken up increasingly deeper by roots, or that their rings were formed earlier in spring and later in autumn (phenological shift linked to climate change).

According to intra-annual isotopic variations in tree rings, aridity of the climate in May forced trees to tap into deep- water sources poor in  $^{18}\text{O}$  (figure 4b, p. 65). The increase in the  $\delta^{18}\text{O}$  signal between September and October attests to the gradual return to more superficial water uptake from autumn rains.

Studies of ring widths in different Mediterranean regions from Cyprus to rural Provence (France) (data not shown) confirm these results. When aridity increases, annual ring growth is correlated with the climate of the 3-5 previous years, which suggests that the available water comes from increasingly deeper soil layers, where the stock depends on several years' cumulative rainfall.

FIGURE 4

**TIME-SERIES OF OXYGEN ISOTOPIC COMPOSITION ( $\delta^{18}\text{O}$ )  
IN TREE RINGS OF ALEPPO PINE (SARRIS *et al.*, 2013),  
ABOVE FROM 1972-2000 (5-YEAR MEANS) WITH MEAN ANNUAL TEMPERATURE ( $^{\circ}\text{C}$ )  
AND RAINFALL (mm) AND BELOW, IN MICRO-SEGMENTS OF RINGS  
CORRESPONDING TO WOOD FORMED EACH MONTH DURING A GROWING SEASON**



### Depth of nutrient uptake in Brazilian eucalyptus plantations

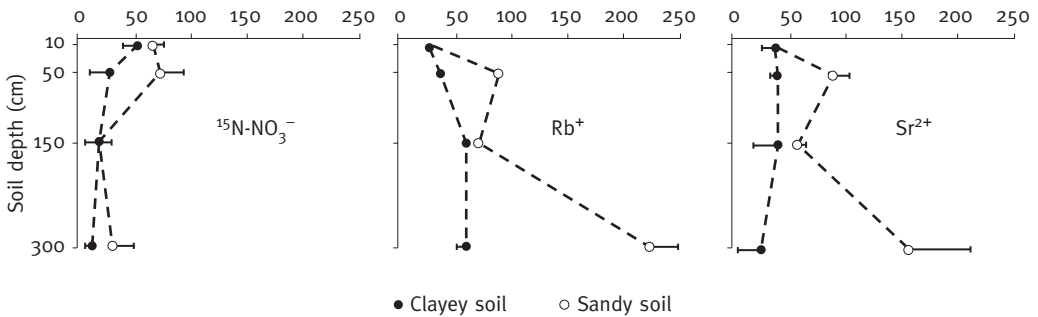
Eucalyptus plantations were studied to gain insight into uptake by deep roots of water and nutrients leached from upper soil layers. Fertilisation is necessary to maintain the high production of these plantations (around  $50 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ ) and the amount of fertiliser that can be applied while avoiding deep drainage losses is an important economic issue.

Labelling occurred twice on two soils with contrasting textures (sandy vs clayey). Solutions labelled with  $^{15}\text{N}$ -enriched nitrate, Rb (K analogue) and Sr (Ca analogue) were applied to the soils using polyethylene pipes at depths of 10, 50, 150 and 300 cm. Care was taken to avoid polluting the upper soil layers. Tracers were applied at least 30 m from each other to decrease the risk that the same root would take up multiple tracers. A root-activity index was calculated for each soil depth from the concentration of each element measured in neighbouring tree leaves 70 days after application. This index was then related to mean root length at the same soil depth to estimate the potential root specific activity, expressed per unit of root length, an indicator of the amount of tracer absorbed at each soil depth (figure 5, p. 66).

Even though  $^{15}\text{N}$ , Rb and Sr were applied at the same time and the same place, the ability of fine roots, calculated per unit length, to take up Rb in the deeper soil layers (and also Sr in the sandy soil), was greater than the ability to take up nitrates, whose uptake was similar at all depths. These results suggest a functional specialisation of deep roots for taking up cations and confirm previous



**FIGURE 5 POTENTIAL ROOT SPECIFIC ACTIVITY (PRSA, INDICATOR OF THE AMOUNT OF TRACER ABSORBED AT EACH SOIL DEPTH, EXPRESSED PER UNIT OF ROOT LENGTH) 70 DAYS AFTER APPLICATION OF TRACERS ON A EUCALYPTUS PLANTATION AT THE END OF A ROTATION IN BRAZIL (% cm<sup>-1</sup>.cm<sup>3</sup>)**

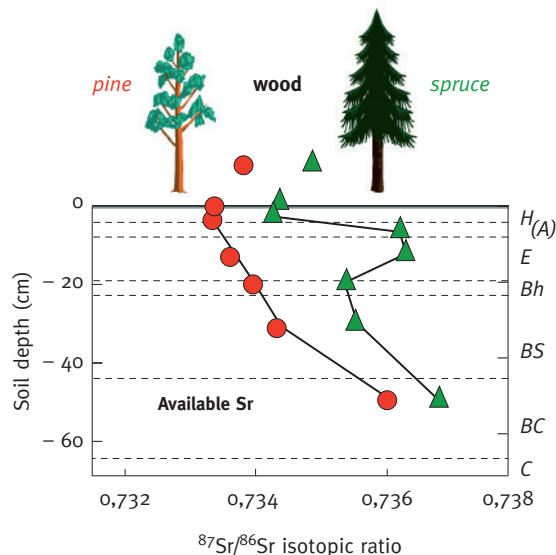


observations of excised roots of beech, oak and Norway spruce. The results of this study and complementary studies that quantified nutrient losses through drainage (Laclau *et al.*, 2010) led to a significant decrease in the amount of fertiliser applied to hundreds of thousands of hectares of eucalyptus plantations in Brazil.

#### Depth of nutrient uptake in a boreal-forest ecosystem

Sr is a trace element analogous to Ca, and the study of natural  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic ratios enables estimation of Ca sources in an ecosystem. In a mixed population of pine and spruce trees on podzols in northern Sweden, the K-rich rocks have a high  $^{87}\text{Sr}/^{86}\text{Sr}$  signature ( $\approx 0.75$ ), whereas the signature of rain is significantly lower ( $\approx 0.71$ ). An isotopic gradient that increases with depth exists in the soil due to the decreasing contribution of Sr from rainfall. In this study (Poszwa *et al.*, 2004), the gradients measured under pine and spruce (figure 6) were modelled and explained by (i) the

**FIGURE 6 COMPARISON OF THE ISOTOPIC COMPOSITION OF STRONTIUM (Sr) (A Ca ANALOGUE) IN SOILS AND TREES (PINE AND SPRUCE) IN SWEDEN TO ESTIMATE THE DEPTH OF NUTRIENT UPTAKE BY ROOTS**



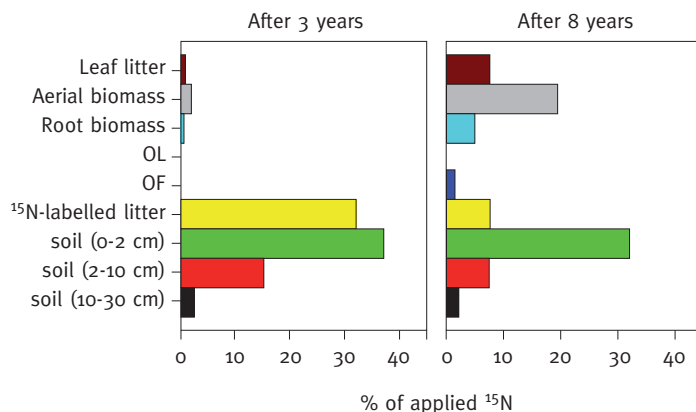
greater return of Sr to the soil via the annual fall of spruce needles (more abundant than that from pine trees, with higher Ca and Sr concentrations than pine needles) and (ii) specific weathering under spruce (*i.e.* a radiogenic peak in the E horizon of the podzol). The Sr isotopic composition in the vegetation reflects the Sr taken from the soil, which makes it possible to estimate the mean depth at which trees take up Sr, and consequently that for Ca. Pines take up Sr and Ca at a depth of approximately 20 cm, whereas spruces take them up at a depth of approximately 10 cm. These results show that mineral uptake in the two species remains relatively shallow but slightly deeper for pines. They supplement a previous study of the same population using O and H isotopes that concluded that pines had deeper water uptake.

## PROCESSES IN SOILS

### Fate of nitrogen from labelled litter spread on the forest floor

N is often a limiting nutrient for both tree growth and soil C sequestration. Leaf litter is the main N source for non-fertilised forest soils.  $^{15}\text{N}$ -enriched beech leaves were used to better understand N cycling in a young beech stand (Ebrach forest experimental site, Germany). Leaf litter was quickly colonised and transformed by both soil fauna and soil fungi. After less than 1 year, fungi had assimilated about 15% of N derived from litter, mainly labile compounds. After the same period, herbivory and soil eating-fungi had assimilated about the same amount of these labile compounds. Three years after litter-fall, the combined activity of soil fauna and transfer processes led to (i) a small (2-4%) but rapid N uptake by trees (mainly as mineral compounds) and (ii) rapid transfer of 55% of N from litter to soil surface layers (Zeller *et al.*, 2001) (figure 7). This transfer mainly occurred as litter fragments were preferentially mineralised by soil microorganisms, whose activity increased N bioavailability for trees. Eight years after litter-fall, 30% of its N was again taken up by trees and mainly allocated to aboveground tree compartments (figure 7). Similarly, forest-floor fragments gradually aggregated on contact with soil organo-mineral complexes (Hatton *et al.*, 2012). N transfer to stable aggregates prevents its mineralisation by microorganisms, ensuring its own retention and that of the associated C. Monitoring the fate of litter N at the nanoscale highlighted the role of microorganisms in forming and retaining organic matter in the form of organo-mineral complexes

**FIGURE 7 DISTRIBUTION OF A COHORT OF  $^{15}\text{N}$  IN THE YOUNG BEECH FOREST OF EBRACH (GERMANY), 3 AND 8 YEARS AFTER THE DEPOSITION OF  $^{15}\text{N}$ -ENRICHED LEAVES TO THE SOIL SURFACE. NEW BEECH LEAF LITTER WAS COLLECTED AFTER LABELLING WITH  $^{15}\text{N}$  AND REPLACED BY NON-ENRICHED LITTER**

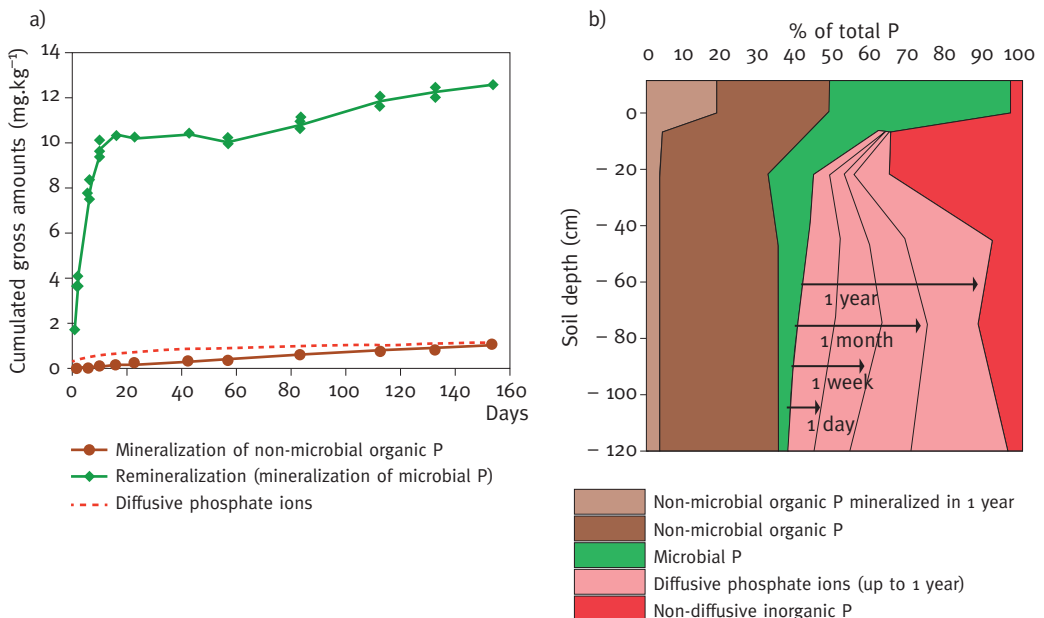


stable at a decadal scale. This  $^{15}\text{N}$  enrichment experiment identified mechanisms of N redistribution and quantified their fluxes in multiple compartments of contrasting forest ecosystems. In the long-term, this kind of approach will enable development of new management practices, optimise bioavailability of soil N for trees and increase the soil's ability to stock C.

### Phosphorus availability in forest soils

P is a limiting nutrient for forest growth; however, the processes governing P availability remain poorly understood. An experiment used isotopic labelling with  $^{32}\text{P}$  and  $^{33}\text{P}$  to study P availability in highly P-deficient soils of the forest range of the *Landes de Gascogne* (southwest France), under maritime pine (*Pinus pinaster*) plantations (figure 8). Processes that replenish phosphate ions in the soil solution include (i) physico-chemical processes (diffusion at the solid-to-solution interface), which depend on inorganic forms of P, and (ii) microbial processes (mineralization of non-microbial organic P and turnover of P in soil microorganisms). Measuring diffusive phosphate ions in soil suspensions over time describes fast (related to surface reactions) and slow reactions (related to the porosity of the solid phase) (figure 8a). These reactions, identified using labelling with  $^{32}\text{P}$  and isotopic dilution, can be predicted from soil physico-chemical properties (Achat *et al.*, 2011). Flux dynamics of organic-P mineralization and re-mineralization of microbial P can be assessed by combining soil incubation under controlled conditions with  $^{33}\text{P}$  labelling (Achat *et al.*, 2009) (figure 8a). In the study at the forest range of the *Landes de Gascogne*, the relative contribution of microbiological processes to P availability decreased with increasing soil depth, compared to physico-chemical processes (diffusion) (figure 8b).

**FIGURE 8**  
**(a) FLUX OF PHOSPHATE IONS AT THE SOLID-SOLUTION INTERFACE**  
**IN A SAMPLE FROM THE SOIL SURFACE LAYER (0-15 CM),**  
**QUANTIFIED IN BATCH EXPERIMENTS AND INCUBATIONS USING  $^{32}\text{P}$  AND  $^{33}\text{P}$  TRACERS.**  
**(b) CONTRIBUTIONS OF ORGANIC- AND INORGANIC-P FRACTIONS, EXPRESSED AS A PERCENTAGE**  
**OF TOTAL P FOR SAMPLES TAKEN AT DIFFERENT SOIL DEPTHS**  
**(EXAMPLE FROM PINE PLANTATIONS OF LANDES DE GASCOGNE, FRANCE)**

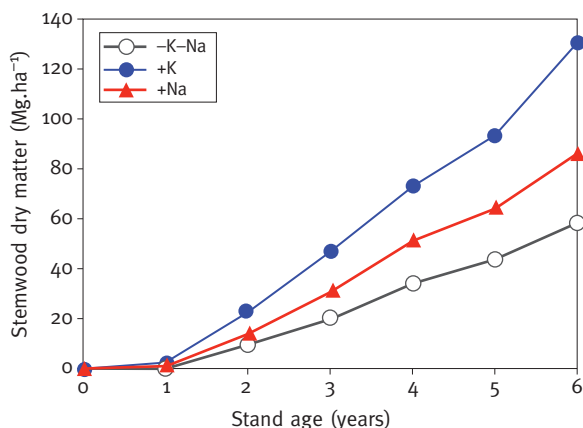


## Nutrient substitution in eucalyptus plantations

Eucalyptus plantations in the Congo that responded to potassium (K) fertilisers much less than plantations with similar soil K-stocks in other regions led to the hypothesis that sodium (Na) can partially replace K in the physiology of eucalyptus trees (substitution between these elements has been described in the literature for other plant species). An experiment was set up in Brazil to test the hypothesis that high atmospheric deposition of NaCl in coastal areas allows eucalyptus to partially substitute K with Na and thus increase wood production (Almeida *et al.*, 2010). A control treatment without K or Na input was compared to treatments receiving either K or Na, or a mixture of both. All treatments were fertilised with other nutrients to study the specific effect of K and Na.

NaCl application increased harvested wood by more than 30 t of dry matter ha<sup>-1</sup> compared to the control treatment (figure 9). NaCl application increased Na concentrations in above ground biomass but did not change K concentrations. Partial substitution of K with Na in tree physiology may explain this response (Battie-Laclau *et al.*, 2014). These results suggest that K fertilisers do not need to be purged of Na. They also explain the relatively high biomass production observed in coastal plantations receiving no fertiliser but high atmospheric deposition of marine aerosols. Experiments are currently underway to explore replacing the current supply of pure KCl fertiliser with a mixture of KCl and NaCl to take advantage of the low cost of NaCl.

**FIGURE 9 WOOD BIOMASS PRODUCTION OVER AN ENTIRE ROTATION OF A EUCALYPTUS PLANTATION IN BRAZIL (HARVESTING 6 YEARS AFTER PLANTING). COMPARISON BETWEEN THE CONTROL TREATMENT RECEIVING NO POTASSIUM OR SODIUM (-K-Na), THE TREATMENT FERTILISED WITH KCl (+K), AND THE TREATMENT FERTILISED WITH NaCl (+Na, WITHOUT ADDITION OF K)**



## CONCLUSION

Recent studies illustrate the appeal of using geochemical and isotopic tracers to improve knowledge about bioavailability of water and elements, soil fertility and plant nutrition. Depending on the context and the tracer(s), these approaches define sources, estimate the residence time of water and elements, trace fluxes of nutrients and identify processes that control biogeochemical cycles.

The approaches presented in this article may not completely replace more conventional approaches (*e.g.* flux measurements, mass balance, input-output budgets). These new tools are powerful, but sometimes difficult and expensive to implement; therefore, they should be used only when conventional approaches fail to meet objectives. When sources of nutrients or elements have sufficiently distinct geochemical or natural isotopic compositions, it is possible to exploit these differences. The natural

abundance approach assesses fluxes only in systems in equilibrium, where the sources are few (ideally two), and when rigorous flux measurement and modelling are performed. It is not so effective when sources have similar or multiple signatures, or when systems are not in equilibrium. Artificial enrichment is complementary and consists of creating an isotopically enriched pool of element(s) and measuring its isotopic dilution in the ecosystem over time. It is now widely used by the scientific community for N, C, H, O and has progressively increased for other elements such as Mg and Ca.

Combination of approaches of natural and enriched abundance, and the simultaneous use of different isotopic systems (C, N, O, Ca, Mg) and/or geochemical tracers (Li, B) coupled with *ex situ* and *in situ* experiments will overcome a number of obstacles in the near future and will respond, at least in part, to the questions raised in the introduction. For example, the  $^{18}\text{O}$  signature used to estimate the depth of water uptake in the Mediterranean region could be used in other regions and consider the phenological shift in growth due to climate change. Mg isotopes may be useful tracers of functional processes, for example, to quantify internal Mg translocation in trees on poor soils. Monitoring changes in enriched litter through  $^{13}\text{C}$  and  $^{15}\text{N}$  (or other tracers) will refine understanding of decomposition and stabilisation of soil C. Use of *in situ* multi-element tracing ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) on eucalyptus plantations in Brazil submitted to artificially limited rainfall will improve understanding of element storage/remobilisation in trees and partition between above or belowground compartments under different environmental conditions. Further studies could be conducted on the relationships between diffusive phosphate ions (described using  $^{32}\text{P}$  labelling and isotope dilution) and soil properties. Isotopic tools are highly useful for understanding soil functioning and identifying sources and dynamics of elements, which is the basic knowledge necessary for sustainable management of soil and forest-ecosystem functions.

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## ISOTOPIC APPROACH TO TRACE WATER AND NUTRIENT DYNAMICS IN FOREST SOILS (Abstract)

Forest soil fertility is generally estimated by studying the water and nutrient cycles essential to living organisms (*i.e.* biogeochemical cycles). Use of geochemical and/or isotopic tracers is an innovative, complementary approach to more traditional studies. Experimental procedures and results of some recent studies in forest ecosystems using natural tracers (*e.g.*  $^{18}\text{O}$ ,  $^{13}\text{C}$ ,  $^{26}\text{Mg}$ ) or artificially supplied tracers (*e.g.* enrichment in Sr, Rb,  $^{15}\text{N}$ ,  $^{44}\text{Ca}$ ,  $^{26}\text{Mg}$ ,  $^{32}\text{P}$ ) are presented. Results are discussed in terms of relevance for using these tools to identify sources, to estimate residence times of water and elements, and to trace nutrient fluxes, of both organic and mineral origins, that are internal or external to the ecosystem.

