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ABSTRACT

In productive grasslands, management practices can alter root mass investment, e.g. extensive management (low cutting frequency, low N fertilisation) induced higher root accumulation than for intensive one. Change of root morphology may have consequences on root distribution of length, root resources use or root longevity. We report response of seasonal root mass variation and root functional traits to cutting frequency (three and six cuts per year, C- and C+, respectively) and N fertilisation (120 and 360 kg N ha^{-1} yr^{-1}, N- and N+, respectively) of 13 grass species grown for three years in field situation as monocultures. Root mass of the 0-15cm depth increased in C- and N- treatments, 10% and 22%, respectively. These effects were more pronounced in spring in comparison with summer and autumn, +44% and +53% for the response to cut and N fertilisation, respectively. Primary roots of C- treatment had significantly higher diameter (+27%) and consequently lower specific root length (SRL, -16%) than C+ treatment. Finest roots (2nd and 3rd order) of N+ exhibited significant lower tissue density (TD) and diameter and higher SRL values than N- treatment. These results emphasise plasticity of root functional traits of grasses in response to cutting and N fertilisation. Extensive treatments favoured higher root allocation and root traits syndrome related to conservation of nutrient, whereas intensive management induced root trait values related to exploitative strategy.

KEYWORDS: Grass, root functional traits, cut, N fertilisation, root types

INTRODUCTION

In productive grasslands, management practices can alter root mass production, e.g. extensive management (low cutting frequency, low N fertilisation) increased root mass in comparison with more intensive one (Klump et al 2007). Plasticity of root morphology has been observed in response to N fertilisation for grasses (Ryser & Lambers 1995; Ryser 1998) which may have important consequences on root distribution of length, root resources use or root longevity. More recently, there are increasing interests to describe plant root system as an integration of multiple genetically and anatomically determined functional root classes (Waisel & Eshel, 2002; Pierret et al 2007). Grass root system is built hierarchically, as diameter generally increases with decreasing order of branching thus diameter distribution of a root system is associated with its branching pattern and variation in root topology has consequences for construction cost per unit root length (Ryser 1998). Thus attention should be given to the distinction in physiological traits that exists among root types. In the present study, we report response of root mass seasonal variation and root functional traits to cutting frequency (three and six cuts per yr, C- and C+, respectively) and N fertilisation (120 and 360 kg N ha^{-1} yr^{-1}, N- and N+, respectively) of 13 grass species grown for three years in field situation as monocultures. In extensive (C-N-) managements, we hypothesise concurrent higher root mass and root traits syndrome related to conservative strategy, whereas in intensive management (C+N+), a decrease of root mass and root traits syndrome related to exploitative strategy.

MATERIAL AND METHODS

The field experiment was located in an upland area of central France at Theix (45°43'N, 03°01'E, 870m a.s.l.) on a granitic brown forest soil (Cambic soil, FAO). In 2001, monocultures of 13 grass species were sown in rows (2m length), 14cm apart, in plot of 4.2m^2 each. The complete experimental design included 168 monocultures arranged in a randomized block design, with three blocks per species and treatment (Pontes et al. 2007a). The 13 co-occurring grass species were representative of fertile and productive

grasslands of French Massif Central: *Arrhenatherum elatius*, Ae; *Anthoxanthum odoratum*, Ao; *Alopecurus pratensis*, Ap; *Dactylis glomerata*, Dg; *Elytrigia repens*, Er; *Festuca arundinacea*, Fa; *Festuca rubra*, Fr; *Holcus lanatus*, Hl; *Lolium perenne*, Lp; *Phleum pratense*, Php; *Poa pratensis*, Pp; *Poa trivialis*, Pt; *Trisetum flavescens*; Tf. From spring 2002 to autumn 2004, these monocultures were subjected to six and three cuts each year, at a six cm height above stem base and were supplied with nitrogen fertilisation (NH_4NO_3, 120 and 360 kg ha^{-1} year^{-1}) applied after each cut, to ensure non-limiting nitrogen nutrition. Supplemental watering was supplied in summer 2003 and 2004 to increase soil volumic water content above 10%.

Root collection and washing

In spring, summer and autumn 2004, root cores (15cm depth, 8cm diameter) were collected with an auger beneath the vegetation and then stored at -18°C before subsequent processing. The core samples were defrosted, cleaned to remove soil from roots and washed with tap water through sieves of 1 and 0.2mm (Picon-Cochard et al 2009). The samples were then oven-dried (48h at 60°C) and weighed. Root mass, including live and dead roots, was expressed as gDM m^{-2}.

Root morphology

After the washing process, subsamples of fresh roots of autumn samples were stained with methylene blue (5 g l^{-1}) during one night at 5°C to increase contrast for scanning. Four subsamples were separately scanned: two subsamples representing all root types of the samples, one subsample representing the finest roots (2nd and 3rd order roots), and one subsample representing the coarsest ones (primary roots). We checked that all sub-sampled roots had no cortex sloughing to avoid any artefact for diameter measurement. For each subsample, fresh coloured roots were spread over a wet mesh screen, transferred to a transparent acetate sheet and then scanned with a transparent light system at a resolution of 400dpi (Epson 1600XL Pro). The WinRhizo software (V2002c, Régent Instruments, Ca) was used to determine root length (m), average root diameter (D, mm), root length and volume (V, cm^3) by class diameter (10 classes of 0.1mm). Thereafter each four subsamples were oven-dried (48h at 60°C) and weighed to calculate root tissue density (TD, g cm^{-3}), specific root length (SRL, m g^{-1}) and root fineness (L/V, cm cm^{-3}). For each root trait, mean of the two sub-samples of all roots was calculated and used as one value.

RESULTS AND DISCUSSION

Seasonal trend of root mass was observed both in intensive and extensive treatments (C+N+, C-N-) but with different patterns (Fig 1). In C+N+, root mass significantly (P<0.05) doubled from spring to summer and declined by 32% in autumn, while in C-N-, root mass decreased by 22% (P<0.05) from spring to autumn. Both cutting frequency and N fertilisation significantly (P<0.05) affected root mass: +10% for C- in comparison with C+ and +22% for N- in comparison with N+.
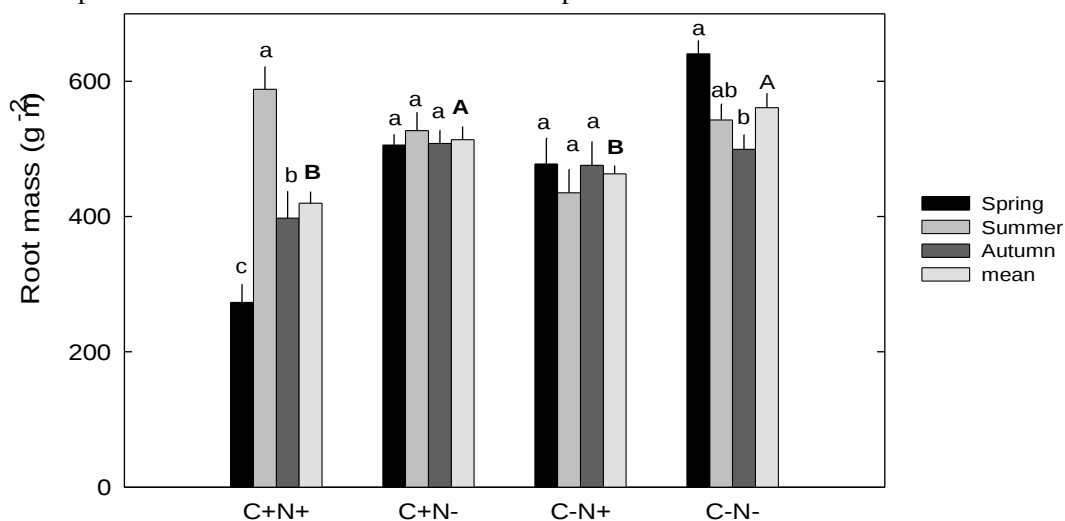
Figure 1: Spring, summer, autumn and mean values of root mass (0-15cm, g m^{-2}) of 13 grass species subjected to six or three cuts per year, C+ and C-, respectively, and to 360 or 120 kg N ha^{-1} year^{-1}, N+ and N-, respectively. Vertical bars correspond to standard error; lower and upper case letters correspond to significant differences (P<0.05) between seasons for each treatment and between treatments whatever the season, respectively.

Reducing cutting frequency (C-) increased total root length by 20%, with a markedly effect for N+ treatment (+60%), while N- treatment exhibited higher root length than N+ (+43%) but only for C+ treatment. Possibly different processes should explain responses to cut and N. In low cutting frequency treatment, competition for light selects taller plants having higher root investment in mass and length that are more productive (Klump et al 2007; Maire et al 2009). In low N treatment, the increase of root mass and root length may improve the efficiency of N acquisition to match plant N demand.

Species was the main factor of root traits variation (Table 1), as observed for leaf traits for the same experiment (Pontes et al 2007a). *D. glomerata* (Dg) exhibited higher variation of root trait values in response to cut and N fertilisation, whereas *F. arundinacea* (Fa), *F. rubra* (Fr) and *A. elatius* (Ae) had the least response to both treatments. In the case of Dg, decreasing cutting frequency induced higher coarse root diameter (D, +31%) and higher fine root density (TD, +52%), while decreasing N fertilisation declined mean D by 11%, but slightly increased fine root D and increased fine root TD by 56% (Fig 2). Whatever the species, we observed significant interaction between cutting frequency and N fertilisation, meaning that response to N of root traits was modulated by frequency of cut.
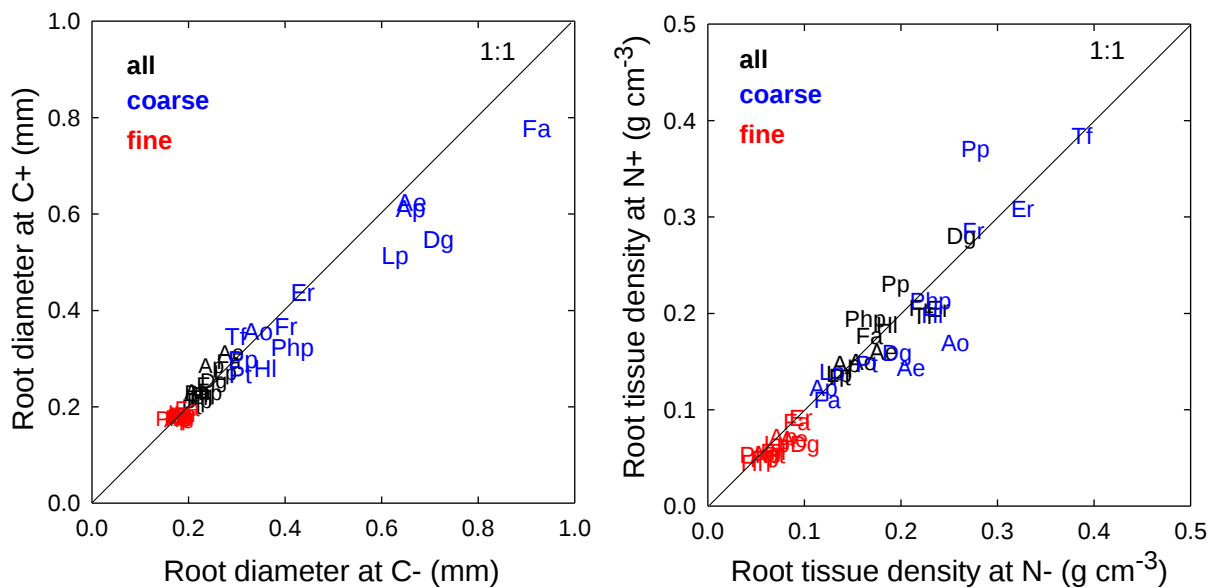

Figure 2: Relationships between diameter measured at C+ and diameter measured at C- (left figure) and between tissue density measured at N+ and tissue density measured at N- (right figure), for 13 grass species. Ae: *Arrhenatherum elatius*, Ao: *Anthoxanthum odoratum*, Ap: *Alopecurus pratensis*, Dg: *Dactylis glomerata*, Er: *Elytrigia repens*, Fa: *Festuca arundinacea*, Fr: *Festuca rubra*, Hl: *Holcus lanatus*, Lp: *Lolium perenne*, Php: *Phleum pratense*, Pp: *Poa pratensis*, Pt: *Poa trivialis*, Tf: *Trisetum flavescens*. Blue, black and red letters correspond to coarse, all and fine roots, respectively. Line corresponds to 1:1.

For traits measured on all root types, most of them were not modified by cut and N treatments. However, significant cut treatment effect was mainly observed on coarse roots (D, SRL, L/V), whereas N fertilisation affected traits values of finest roots (D, TD, SRL) (Table 1, Fig 2). Higher coarse root D under low cutting frequency is consistent with the fact that plants are taller under this treatment (Kutschera & Lichtenegger 1982; Ryser 1998; Wahl & Ryser 2000). In response to N fertilisation, finest roots (2nd and 3rd order) of N+ exhibited significant lower TD and D and higher SRL values than N- treatment, syndrome of traits related to exploitative strategy.

CONCLUSIONS

In this study we confirmed that extensive and intensive treatments favoured higher and lower root allocation and root traits syndrome was related to conservation and exploitation, respectively. These results also emphasised plasticity of root functional traits of grasses in response to cut frequency and N fertilisation for coarse and finest roots, respectively, which correspond in grasses to primary roots and to second and third order roots. Obviously the use of mean root trait values was not sufficient to highlight

root plasticity to cut and N treatments. Finally, these results stressed that response of grass root traits to cut and N were not independent and this should be considered before any generalisation could be done.

Table 1: Root traits of 13 grass species monocultures subjected to six (C+) or three (C-) cuts per year and to two levels of N fertilisation (360 and 120 kg N ha^{-1} yr^{-1}, N+ and N-, respectively), measured in autumn for each treatment and for all root types, coarse and fine roots. D is diameter (mm), TD is tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), L/V is fineness (cm cm^{-3}). Values are mean of 39 plots, with 3 replicates by species. For each trait and root type, different letter indicate significant differences at P<0.05. Last six columns correspond to ANOVA analysis for species, cut, N, species x cut, species x N and cut x N interactions. NS: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001.

Traits	Root type	C+N+	C+N-	C-N+	C-N-	Species	cut	N	Species x cut	Species x N	Cut x N
D	all	0.257 a	0.234 b	0.229 b	0.254 a	***	NS	NS	NS	NS	***
	coarse	0.461 bc	0.422 c	0.483 ab	0.511 a	***	***	NS	NS	NS	*
	fine	0.182 b	0.178 c	0.176 c	0.187 a	***	NS	***	*	*	***
TD	all	0.186 a	0.193 a	0.184 a	0.178 a	***	NS	NS	NS	NS	NS
	coarse	0.204 a	0.233 a	0.218 a	0.218 a	***	NS	NS	NS	NS	NS
	fine	0.067 b	0.063 bc	0.058 c	0.083 a	***	*	***	NS	NS	***
SRL	all	141 c	160 b	181 a	136 c	***	NS	*	NS	NS	***
	coarse	40 a	38 a	33 b	33 b	***	***	NS	***	**	NS
	fine	692 b	737 ab	804 a	564 c	***	NS	***	NS	NS	***
L/V	all	2471 c	2788 b	3013 a	2279 c	***	NS	**	*	*	***
	coarse	843 a	823 a	687 b	747 ab	***	**	NS	NS	NS	NS
	fine	4207 ab	4241 a	4311 a	4058 b	***	NS	NS	NS	NS	*

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