

# Root trait responses of six temperate grassland species to intensive mowing and NPK fertilisation: a field study in a temperate grassland

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## Abstract

**Background and aims** Plant traits may characterize functional ecosystem properties and help to predict community responses to environmental change. Since most traits used relate to aboveground plant organs we aim to explore the indicative value of root traits.

**Methods** We examined the response of root traits (specific root length [SRL], specific root surface area [SRA], root diameter [RD], root tissue mass density [TMD], root N concentration) in six grassland species (3 grasses, 3 herbs) to four management regimes (low vs. high mowing frequency; no fertilization vs. high NPK fertilization). The replicated experiment in temperate grassland with long continuity simulated the increase in grassland management intensity in the past 50 years in Central Europe.

**Results** Increasing mowing frequency (one vs. three cuts per year) led to no significant root trait changes. NPK fertilization resulted in considerable trait shifts with all species responding in the same direction (higher SRL, SRA and N concentration, lower TMD) but at

different magnitude. Fertilization-driven increases in SRA were mainly caused by lowered tissue density while root diameter reduction was the main driver of SRL increases.

**Conclusion** We conclude that root morphological traits may be used as valuable indicators of environmental change and increasing fertilization in grasslands.

**Keywords** Dicots · Monocots · Root diameter · Root N concentration · Root tissue density · Specific root length · Trait shift

## Introduction

Leaf morphological traits and the nutritional status of the foliage have widely been used as suitable indicators of plant resource use and growth strategies and for predicting plant responses to environmental change (Díaz and Cabido 2001; Díaz et al. 2004; Reich et al. 1997). In the aboveground compartment, specific leaf area (SLA, leaf area per mass) and nitrogen content per leaf area ( $N_a$ ) were found to be reliable predictors of photosynthetic capacity, growth rate, litter decomposability and other plant functional attributes across life forms, communities and biomes (e.g., Hunt and Cornelissen 1997; Reich et al. 1998a; 2003; Wright et al. 2004). Root traits and their indicative value have been examined to a much lesser extent, which is largely a consequence of the more difficult accessibility of belowground plant organs. The partial ignorance of

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the plant's belowground compartment is a major deficit in many studies on plant functional types, as roots are not only the organs of water and nutrient uptake, but also function as important drivers of soil carbon and nutrient cycling through direct and indirect effects on rhizosphere chemistry (Lukac and Godbold 2011; Fender et al. 2012). Meaningful root traits with a close relation to root physiological activity (nutrient and water uptake) and root longevity are specific root length (SRL, root length per mass) and root N concentration (RNC), in analogy to SLA and  $N_a$  (e.g. Eissenstat et al. 2000; Reich et al. 1998b; Ryser 1996; Tjoelker et al. 2005). In addition, the structure of the rhizodermal (or peridermal, exodermal) tissue may be an indicative trait in the fine roots of trees (Withington et al. 2006). Perhaps the most often investigated root property next to root diameter is SRL (Ostonen et al. 2007), which typically shows a negative relation to root life span (Ryser 1996; 2006; Ryser and Lambers 1995, but see Tjoelker et al. 2005) while it was found to correlate positively with nitrogen uptake rate (Reich et al. 1998b). Specific root area (SRA, root surface area per mass) has only rarely been measured under field conditions; however, it represents the direct belowground analogy to SLA and should even be closer related to exchange processes between root and soil (nutrient and water uptake, rhizodeposition, respiration) than SRL (Mokany and Ash 2008). SRL (and SRA) are determined by the two parameters root tissue mass density (TMD) and root diameter (RD) according to the following equation (Ostonen et al. 2007):

$$SRL = \frac{1}{TDM \times RD^2} \times \frac{4}{\pi}$$

Many grassland ecosystems, in particular those on nutrient-poor and/or drought-affected soils, contain large amounts of root biomass with root:shoot ratios often being much larger than 1 (Gass and Oertli 1980; Fiala 1993; Jackson et al. 1996). However, despite their outstanding importance as a carbohydrate sink in net primary production and for ecosystem carbon cycling, the roots and root systems of grassland plants have only rarely been studied with respect to root morphological traits and their variation with species, environmental conditions, and management regime (e.g. Roumet et al. 2006). One reason is that grasslands are often relatively species-rich and the roots of the different species are not easily distinguishable by morphological

criteria. Further, in comparison to agroforest and forest ecosystems, fine roots (<2 mm in diameter) of grassland herbs and graminoids are typically much thinner than those of trees, and the assessment of root vitality status (live vs. dead) is even more difficult than in tree fine roots (Polomski and Kuhn 1998; Lauenroth and Gill 2003). As a consequence, most studies on the root ecology of grassland plants were conducted in potted cultures under greenhouse conditions (e. g. Craine et al. 2003; Ryser and Lambers 1995) or in sown monocultures (e.g., Tjoelker et al. 2005; Maire et al. 2009), while field studies in established grasslands are scarce (e.g. Milchunas and Lauenroth 1992). However, it is questionable whether root data from potted plants or artificial monocultures can simply be extrapolated to the field situation, as leaf and whole-plant traits from field-grown plants may strongly differ from traits measured ex situ (Mokany and Ash 2008; Poorter and de Jong 1999). One reason for generally poor comparability are different soil conditions; Mokany and Ash (2008) showed that good matching between field-grown and ex situ-grown plants with respect to aboveground traits is dependent on the nutritional status of the plants. We argue that the comparability of trait data between field-grown and potted plants might even be poorer with respect to belowground organs because root systems modify their size, morphology and functioning largely in response to soil physical and chemical conditions (Fitter 1996; Robinson et al. 2003). Further, pot experiments can rarely simulate the belowground competitive situation in grasslands in a realistic way which casts doubt on the more general validity of root trait data collected in ex situ experiments.

While most of Central Europe's grasslands have been managed extensively in the past centuries, post-war grassland management has shifted to highly intensive mowing and grazing regimes which rely on high fertilizer doses (Dierschke and Briemle 2002). Thus, grassland communities are nowadays exposed to much higher disturbance intensities than 40 or 50 years ago, while N, P and K loads and soil compaction in the meadows and pastures have increased. We expect that not only root biomass, root:shoot ratio and root turnover may have changed in response to grassland management intensification but the morphological and physiological root traits of the grassland species as well, reflecting the large increases in nutrient availability, aboveground productivity, disturbance frequency and biomass removal that have taken place.

Frequently mown or grazed plants have been found to reduce their root biomass in order to mobilise more resources for shoot regrowth (Gass and Oertli 1980; Dawson et al. 2000), while root:shoot ratio should increase due to the loss in aboveground biomass. Root longevity might also decline with increasing cutting frequency, as shorter-lived roots should enhance the plants' flexibility to respond to growing stress intensity (Eissenstat and Yanai 1997). Repeated fertilization may stimulate fine root growth as a strategy of the root system to respond to nutrient pulses in ruderals and strong competitors (Craine 2009). This can be achieved by producing more short-lived roots with higher SRL and/or SRA which would allow the species to occupy additional soil space and take up nutrients at reduced carbon and nutrient costs of the root system (Eissenstat 1992; Reich et al. 1998b; Ryser and Lambers 1995; Mokany and Ash 2008; Ostonen et al. 1999). This results in reduced root longevity of grassland plants with increasing nutrient availability (e.g. Craine 2009; van der Krift and Berendse 2002), partly as a consequence of lowered TMD and increased SRL (Elberse and Berendse 1993). However, decreases in SRL with fertilisation and particularly high SRL in plants under nutrient shortage have also been observed (Ostonen et al. 2007; Trubat et al. 2006). Plants exposed to frequent mowing or grazing might similarly respond with increased SRL and/or decreased root tissue mass density (Wahl and Ryser 2000) to these stressors. Marked change in root morphology does not necessarily lead to changes in SRL or SRA, when opposing diameter and density shifts are occurring.

Since a decrease in SRL is also a possible effect of fertilisation (Fitter 1985; Ostonen et al. 2007), it is not clear which response is dominating the root trait reaction in intensively managed grasslands, the fertilisation effect (which may increase or decrease SRL) or the influence of mechanical stress (which should increase SRL). Belowground, cutting and grazing impact only indirectly on the root system which might induce less pronounced responses than the direct fertilizer application. Further, cutting seems to have a weaker effect on aboveground traits of grassland plants than fertilization (Rose et al. 2013). Studies examining how and by which mechanisms (RD or TMD change) SRL and SRA are altered under field conditions in response to environmental or management factors are still lacking.

We investigated five key root traits (SRL, SRA, TMD, RD and RNC) in six abundant plant species (3

herbs, 3 grasses) of a temperate grassland in their response to defined mowing and fertilisation regimes. The study was conducted in the framework of the full-factorial grassland management experiment GrassMan (Petersen et al. 2012) in a grassland with long continuity consisting of two cutting frequencies (1 and 3 cuts per year) and two fertilization levels (0 and 180 kg N-ha<sup>-1</sup> yr<sup>-1</sup>). These management schemes simulate the once widespread low-intensity and the current high-intensity system of grassland management in Central Europe. With this field study, we tested the hypotheses that (i) the five root traits are modified in all six species under a given management regime in the same direction but at different species-specific rates, (ii) the fertilization effect on root morphology is larger than the mowing (cutting) effect, and (iii) both decreases in RD and TMD in response to management intensification are responsible for increases in SRL and SRA. We further searched for evidence that one (or several) of the five root traits can be used as indicators for the species' nitrogen response and sensitivity to cutting based on trait plasticity upon management intensification.

## Material and methods

### Study site, climatic conditions and experimental design

The study was conducted within the framework of the 'GrassMan' project (Petersen et al. 2012), an interdisciplinary study on the role of management regimes and plant diversity for ecosystem functioning in historically-old grassland with more than 200 years of continuity. The site is located on the experimental farm Relliehausen of the University of Göttingen in the Solling Mountains, Central Germany (51°44' N, 9°32' E, 490 m a.s.l.), near the village of Silberborn. The soils are haplic Cambisols on Buntsandstein with a pH<sub>H2O</sub> in the range of 5.2–5.6. Mean annual temperature is 6.9 °C and annual precipitation is 1031 mm (DWD 1961–1990). In the year 2010, a particularly dry period occurred in June and July (26, 47 mm) compared to the long time mean (108, 97 mm for the 2 months, respectively). The terrain is even to slightly sloping (ca. 5°) and covered by homogenous grassland. Before the implementation of the experiment with different management schemes (treatments) in 2008, the site had been used over decades for extensive cattle grazing. The grassland community is a mesic-moist to moist *Lolio-Cynosuretum* (Central European pasture

community) in a montane variety on relatively nutrient-poor soil.

The grassland management experiment was conducted with the intention to examine the consequences of increases in cutting frequency and/or fertilization intensity on a variety of features of permanent temperate grassland including important morphological and functional traits of the key species, the structure and diversity of the community, productivity, and water and nutrient fluxes. For generating an extended diversity gradient and studying assumed diversity effects on ecosystem functioning, selective herbicides were applied to decrease species richness in certain plots by reducing the abundance of either grasses or herbs. The full factorial study design includes three factors. The two different cutting frequencies represent an extensive hay meadow system with one cutting in July (C1), as it was widespread in Central European grasslands 50 years ago, and a more intensive management, where cutting took place three times a year (May, July, and September; C3), being more close to the recent high-input grassland management that dominates today. Half of the plots were NPK-fertilized (NPK+, 180 kg N ha<sup>-1</sup> yr<sup>-1</sup> applied as a mix of NH<sub>4</sub>NO<sub>3</sub> and CaCO<sub>3</sub>, 74:26 w/w, 30 kg P ha<sup>-1</sup> and 107 kg K ha<sup>-1</sup>) and half of them received no fertilizer (NPK-; Table 1). Thus, the low-intensive management treatments (C1 and NPK-) simulate grassland management about 50 years ago, while the high-intensity treatment stands for the recent management regime (C3, NPK+). In the herbicide experiment, one third of the plots was treated with the herbicide Select 240 EC (Stähler Int., Stade, Germany; 0.5 L ha<sup>-1</sup>) that selectively reduced the abundance of monocots (-Mon treatment), one third was treated with the herbicides Starane and Duplosan KV (active components Fluroxypyr/Triclopyr and Duplosan KV; both 3 L ha<sup>-1</sup>) to decrease the cover of dicots (-Dic treatment),

and one third was left as a control with no herbicide application (Co treatment). Biomass yields and the nitrate concentration in the percolating soil water of the treated plots were found to be not affected by the herbicides 1 year after herbicide application (From et al. 2011; Petersen et al. 2012). Further, according to an inventory in spring 2009, herbicide application did not result in disturbance effects such as a decrease in vegetation cover or an increase in the abundance of annual species. The 12 different treatments (2 x cutting, 2 x fertilization, 3 x diversity) were replicated sixfold resulting in 72 plots of 15 m×15 m size arranged in a Latin rectangle with six blocks.

### Root sampling

Sampling was conducted between June 14 and July 16, 2010, after the C3 plots had been cut. We sampled roots of three grass species, *Agrostis capillaris* L., *Dactylis glomerata* L., and *Festuca rubra* L., and three non-legume herbs, *Ranunculus repens* L., *Rumex acetosa* L., and *Veronica chamaedrys* L. (Table 2). Root samples (each one sample per species per plot) were collected in 8 of the 12 treatments (in the herbicide treatments -Dic and Co, while the -Mon treatment was not sampled) with each treatment replicated threefold resulting in 24 plots sampled in total; thus, only every second replicate plot of the experiment (three of the six blocks) was investigated for root traits because the number of root samples that could be processed in due time limited the total number of replicate samples (in our study: 24 plots x 6 species). We used a spade to dig out a chunk of soil (~20–30 cm deep) around the target plant, which was transferred to the laboratory. The root system of the target plant was separated from

**Table 1** Management procedures conducted in the four different treatments of the experiment in the years 2009 and 2010

Procedures						
2009	15/04	14/05	28/05	04/06	09/07	30/09
2010	21/04	26/05	01/06	01/06	20/07	29/09
Treatment	90 kg N ha <sup>-1</sup>	Cutting	90 kg N ha <sup>-1</sup>	30 kg P ha <sup>-1</sup> 107 kg K ha <sup>-1</sup>	Cutting	Cutting
NPK- C1					x	
NPK+C1	x		x	x	x	
NPK- C3		x			x	x
NPK+C3	x	x	x	x	x	x

**Table 2** Species investigated, typical plant height, N indicator value after Ellenberg et al. 1992<sup>a</sup>, and mowing and grazing tolerance scores after Dierschke and Briemle (2002)<sup>b</sup>

Species	Family	Height (cm)	N indicator value	Mowing tolerance
<i>Agrostis capillaris</i>	Poaceae	20–40	4	6
<i>Dactylis glomerata</i>	Poaceae	20–150	5	8
<i>Festuca rubra</i>	Poaceae	20–100	6	9
<i>Ranunculus repens</i>	Ranunculaceae	30–40	7	8
<i>Rumex acetosa</i>	Polygonaceae	30–50	x	6
<i>Veronica chamaedrys</i>	Scrophulariaceae	10–30	5	7

<sup>a</sup> 1 – growing on very N-poor soil, 5 – growing on moderately rich soils, 9 – restricted to soils with very high N availability, <sup>b</sup> 1 – tolerates no mowing, 5 moderately tolerant, 9 insensitive to mowing

other roots by carefully extracting the roots under running water on a sieve (mesh size 0.2 mm). As a minimum, three intact roots per plant individual were cleaned and stored in tap water in a refrigerator (8 °C) until further analyses took place.

#### Morphological and chemical analyses

On a flatbed scanner, we took digital images of the roots with a resolution of 400 dpi; the root branches of a plot and species were pooled. The images were analyzed with the software WinRhizo 2005c (Régent Instruments, Québec, Canada) to calculate the parameters cumulative root length and root surface area, and mean root diameter. All samples were dried (70 °C, 80 h) and weighed ( $\pm 0.1$  mg) to determine specific root length (SRL;  $\text{m g}^{-1}$ ), specific root area (SRA;  $\text{cm}^2 \text{g}^{-1}$ ) and root tissue mass density ( $\text{g cm}^{-3}$ ). The latter calculations were conducted separately for the 11 root diameter classes 0–0.1, 0.1–0.2, 0.2–0.3, 0.3–0.4, 0.4–0.5, 0.5–0.6, 0.6–0.8, 0.8–1.0, 1.0–1.5, 1.5–2.0, and >2.0 mm. Root surface area and volume were summed up in a sample to account for the possibility of non-normal distribution of root diameters, which has the potential to cause severe errors in the estimation of surface area and volume (Ryser 2006). After weighing, all samples were homogenized by cutting them into very small pieces and carefully mixed. Root nitrogen concentrations of a 3 mg subsample were determined with a C/N autoanalyser (vario EL III; elemental Analysensysteme, Hanau, Germany).

#### Statistical analyses

Statistical analyses were performed with the software SAS version 9.1 (SAS Institute, Cary, NC, USA). A

significance level of  $P < 0.05$  was used throughout; results with a significance level of  $0.05 < P < 0.1$  were additionally indicated as trends. All data met ANOVA assumptions.

We calculated general linear models for all data (SRL, SRA, root diameter, tissue density, RNC) of a species. The herbicide treatment (diversity level) had no significant effect on any of the variables and therefore was excluded from the models. Thus, the models included the treatments cutting frequency and fertilization as well as the interaction of both factors as fixed effects. We simplified the models until the remaining factors had a significant impact ( $P < 0.05$ ) or indicated a trend ( $P < 0.1$ ). Cutting frequency as well as the interaction between fertilization and cutting frequency had no significant impact on any of the variables.

Pearson regression analyses were used to describe the relationships between specific root area and specific root length within a species and across the species sample. We used general linear models with sequential sum of squares (Type 1) to describe the dependency of SRL and SRA values on mean root diameter and root tissue density. Models were calculated for every species across all management treatments (intraspecific effects) and across all species with data pooled per species (interspecific effects). Due to a possible intrinsic relation between root diameter and tissue density (which was significant at  $P < 0.05$  in *A. capillaris* and *D. glomerata*), we calculated two alternative models with either diameter or tissue density fitted first (Hector et al. 2010). Additionally, we tested the dependence of root morphology on root N concentration within a species and across the species by linear and non-linear regression analyses, but detected significant relations between SRL or SRA and RNC only in *R. repens* (data not shown).

## Results

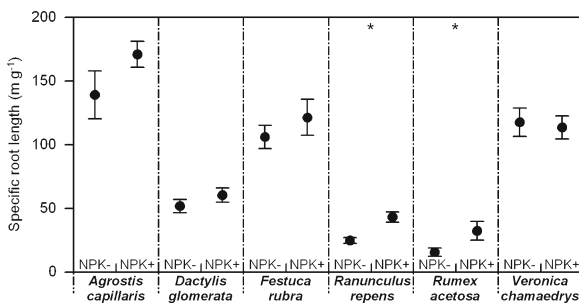
### Among-species variation in root morphology and root N concentration

The mean specific root length of the six investigated species ranged between 24 and 155  $\text{m g}^{-1}$  (minimum: *R. acetosa*, maximum: *A. capillaris*, Fig. 1), specific root area varied between 235 and 860  $\text{cm}^2 \text{g}^{-1}$  (same species extremes, Fig. 2). There was no systematic difference in SRL or SRA values between the monocot and dicot species.

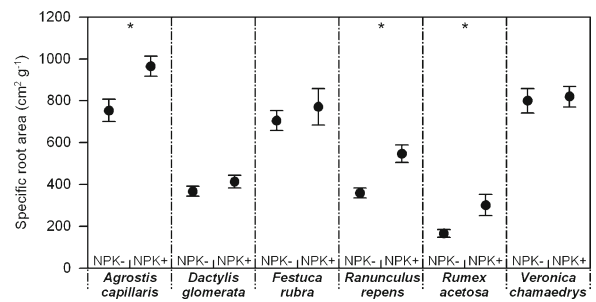
Mean root tissue density ranged between 0.15 and 0.26  $\text{g cm}^{-3}$  in the six species and was lowest in *A. capillaris* and highest in *D. glomerata* (Fig. 3). The species means of root diameter varied between 0.23 (*A. capillaris*) and 0.48 mm (*R. repens*); the dicot species had on average higher root diameters than the monocot species (difference between groups significant at  $P < 0.001$ , Fig. 4). Root nitrogen concentration reached species means between 0.74 and 1.44  $\text{mmol g}^{-1}$  (*D. glomerata* and *V. chamaedrys*, respectively) and was significantly lower in the grass roots than in the herb roots (Fig. 5).

### Management effects on root morphology and root N concentration

*Ranunculus repens* and *Rumex acetosa* were the only species whose SRL values were significantly affected by the management regime (Fig. 1). Both species showed a SRL increase upon NPK fertilization. A significant positive SRA response to NPK fertilization was detected in three species (*A. capillaris*, *R. repens* and *R.*



**Fig. 1** Specific root length of the six grassland species in June/July 2010 in the two fertilization treatments (NPK-: no fertilization, NPK+: 180  $\text{kg N ha}^{-1} \text{yr}^{-1}$ ; means  $\pm$  SE,  $n=10-12$ ). Asterisks denote significant differences between treatments ( $P < 0.05$ )

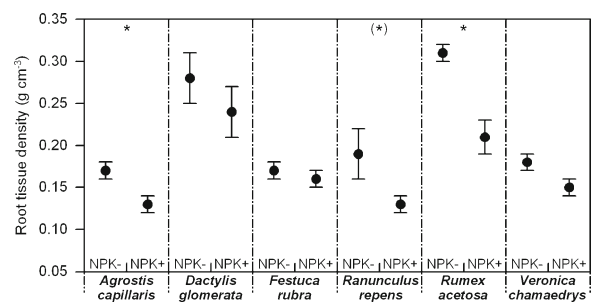


**Fig. 2** Specific root area of the six grassland species in June/July 2010 in the two fertilization treatments (NPK-: no fertilization, NPK+: 180  $\text{kg N ha}^{-1} \text{yr}^{-1}$ ; means  $\pm$  SE,  $n=10-12$ ). Asterisks denote significant differences between treatments ( $P < 0.05$ )

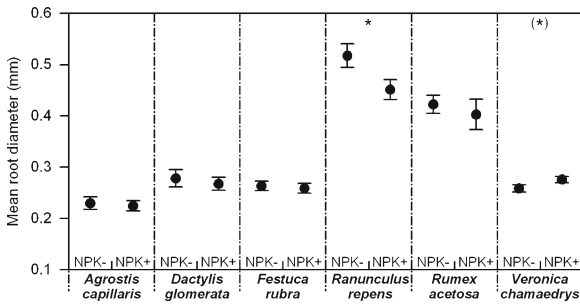
*acetosa*, Fig. 2), while the other three species showed neither a significant response nor a trend towards higher values in fertilized compared to unfertilized plots.

The root tissue mass density of *A. capillaris* and *R. acetosa* was significantly lower in fertilized plots compared to unfertilized plots. In the other four species, marginally significant ( $0.1 > P > 0.05$ ; *R. repens*) or insignificant trends toward a reduced tissue density in the NPK+ treatments were observed (Fig. 3). The only significant effect of the management regime on mean root diameter was a negative response of *R. repens* to fertilization (Fig. 4). In contrast, *V. chamaedrys* showed a marginally significant trend ( $P < 0.1$ ) towards larger root diameters in fertilized compared to unfertilized plots; the root diameter of the other four species remained unaffected by the management.

Root nitrogen concentration was significantly higher after fertilization in *R. acetosa* (increase from 1.04 to 1.67  $\text{mmol g}^{-1}$ , Fig. 5), while *D. glomerata*, *F.*



**Fig. 3** Root tissue density of the six grassland species in June/July 2010 in the two fertilization treatments (NPK-: no fertilization, NPK+: 180  $\text{kg N ha}^{-1} \text{yr}^{-1}$ ; means  $\pm$  SE,  $n=10-12$ ). Asterisks denote significant differences between treatments ( $P < 0.05$ ), asterisks in parentheses stand for marginally significant differences ( $0.1 > P > 0.05$ )



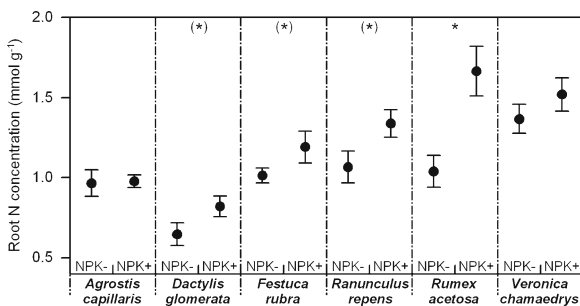
**Fig. 4** Mean root diameter of the six grassland species in June/July 2010 in the two fertilization treatments (NPK-: no fertilization, NPK+: 180 kg N ha<sup>-1</sup> yr<sup>-1</sup>; means ± SE, n=10–12). Asterisks denote significant differences between treatments (P<0.05), asterisks in parentheses stand for marginally significant differences (0.1>P>0.05)

*rubra* and *R. repens* showed trends (P<0.1) towards higher concentrations in fertilized plots. *A. capillaris* and *V. chamaedrys* were not affected by fertilization (Fig. 5).

In contrast to the fertilizer effect, none of the five root traits was significantly influenced by cutting frequency (one vs. three cuts per year: C1 vs. C3 treatment; data not shown).

The inter- and intraspecific relationship between SRL and SRA

SRA and SRL showed the expected positive relation to each other in all species (P<0.001, Table 3). However, the strength of this relationship varied between the species: While the variation in SRL explained 97 % of the SRA variance in *R. acetosa*, only 62 % of the variance was explained in the case of *D. glomerata*.



**Fig. 5** Root nitrogen concentration of the six grassland species in June/July 2010 in the two fertilization treatments (NPK-: no fertilization, NPK+: 180 kg N ha<sup>-1</sup> yr<sup>-1</sup>; means ± SE, n=10–12). Asterisks denote significant differences between treatments (P<0.05), asterisks in parentheses stand for marginally significant differences (0.1>P>0.05)

The SRA - SRL relationship was generally stronger in the dicot than the monocot species (Table 3).

The dependence of SRL and SRA on root tissue density and mean root diameter

Root diameter and root tissue density explained at least 76 % of the intraspecific SRL variance in the six species with root diameter having the larger explicatory power in four of the six species (up to 87 %, Table 4). Exceptions from this rule were *F. rubra* (diameter explained 28 % of the SRL variance) and *V. chamaedrys* (42 %) when diameter was the variable included first in the models, and *R. acetosa* (40 %), when diameter was included after tissue density. No significant impact of tissue density on SRL could be detected in the case of *A. capillaris* and *D. glomerata* when root tissue density was fit first. Root diameter explained more than 50 % of the interspecific SRL variance irrespective of its position in the model (variance explained by models: 97 %, Table 4).

The intraspecific SRA variance was explained to at least 70 % by root tissue density and mean root diameter, and root tissue density was the variable explaining the greater proportion (42–71 %; minimum: *D. glomerata*, maximum: *V. chamaedrys*) for all species except for *A. capillaris*, when it was fit first, and for *R. acetosa*, when fit as the second explanatory variable (Table 4). Root diameter had no significant effect on SRA in *D. glomerata* and *F. rubra* when it was fit first. Ninety-nine percent of the interspecific SRA variance was explained by the models that included root tissue density and root diameter (Table 4). However, the proportion of variance explained by these variables depended on their position in the model: when fitted first, tissue density explained more SRA variance than root diameter (66 %) but more or less the same amount of variance (49 %), when it was included as the second variable (Table 4).

## Discussion

We found consistent root trait alterations in all six grassland species in response to increasing management intensity. While the direction of change was the same for all species for a given trait (with one exception), the magnitude of trait change was different among the species, supporting our hypothesis (1). The roots of the

**Table 3** Summary of regression analyses on the dependence of SRA on SRL for the six species and all species pooled (all species)

	Intercept			SRL			Model	
	Estimate	Error	<i>P</i>	Estimate	Error	<i>P</i>	R <sup>2</sup>	<i>P</i>
<i>A. capillaris</i>	361.66	67.43	***	3.21	0.41	***	0.73	***
<i>D. glomerata</i>	160.15	41.34	***	4.11	0.70	***	0.62	***
<i>F. rubra</i>	122.70	57.26	*	5.52	0.48	***	0.87	***
<i>R. repens</i>	125.61	31.87	***	9.59	0.86	***	0.86	***
<i>R. acetosa</i>	69.90	8.11	***	6.83	0.25	***	0.97	***
<i>V. chamaedrys</i>	210.08	39.40	***	5.18	0.33	***	0.92	***
All species	194.13	69.31	*	4.66	0.72	**	0.91	**

\*\*\*, \*\*, \*, *P*<0.001, 0.01, 0.05, respectively

dicot species tended to be more plastic in their morphological response to increasing fertilization (7 significant and 3 marginally significant trait shifts in the species x trait matrix) than the roots of the monocot species (2 significant and 2 marginally significant trait shifts). Specific root length and area, tissue mass density and root N concentration revealed larger relative alterations upon NPK fertilization in the dicots *R. repens* and *R. acetosa*

than in the three monocot species; the third dicot species *V. chamaedrys* had a similarly small plasticity as the monocots.

An unexpected result is that none of the species altered its root morphology in response to an increase in mowing frequency from one to three cuts per growing season, while more frequent cutting reduced the standing fine root biomass in the upper soil (0–15 cm)

**Table 4** Summary of general linear models on the dependence of SRL or SRA on root tissue mass density and mean root diameter

	Intercept		Root tissue density						Mean root diameter						Model	
	E	SE	E	SE	% Ve <sup>a</sup>	<i>P</i> <sup>a</sup>	% Ve <sup>b</sup>	<i>P</i> <sup>b</sup>	E	SE	% Ve <sup>a</sup>	<i>P</i> <sup>a</sup>	% Ve <sup>b</sup>	<i>P</i> <sup>b</sup>	R <sup>2</sup>	<i>P</i>
SRL																
<i>A. capillaris</i>	611	43	-752	129	0	ns	22	***	-1520	131	87	***	65	***	0.87	***
<i>D. glomerata</i>	201	18	-141	25	2	ns	36	***	-392	49	75	***	41	***	0.77	***
<i>F. rubra</i>	487	49	-698	113	23	***	49	***	-990	151	54	***	28	***	0.77	***
<i>R. repens</i>	119	11	-173	38	31	***	25	***	-124	21	45	***	51	***	0.76	***
<i>R. acetosa</i>	125	11	-125	29	41	***	17	***	-166	25	40	***	63	***	0.80	***
<i>V. chamaedrys</i>	568	29	-754	63	25	***	50	***	-1230	89	67	***	42	***	0.93	***
All species	307	24	-528	102	43	**	27	*	-381	52	54	**	70	**	0.97	**
SRA																
<i>A. capillaris</i>	2689	158	-4932	479	24	***	67	***	-4862	486	63	***	20	***	0.87	***
<i>D. glomerata</i>	1002	107	-1021	151	42	***	69	***	-1241	290	28	***	0	ns	0.70	***
<i>F. rubra</i>	2521	328	-4822	752	49	***	67	***	-3731	1008	22	**	4	ns	0.71	***
<i>R. repens</i>	1193	121	-2575	417	57	***	53	***	-765	224	16	**	21	**	0.74	***
<i>R. acetosa</i>	919	77	-1116	201	53	***	29	***	-958	176	27	***	52	***	0.81	***
<i>V. chamaedrys</i>	3025	172	-4870	382	48	***	71	***	-5299	538	43	***	19	***	0.91	***
All species	1713	77	-3483	324	66	**	49	**	-1437	167	32	**	50	**	0.99	**

<sup>a</sup>Root tissue density fit first, <sup>b</sup>Root diameter fit first. All species pooled: All species

*E* Estimate, *SE* Standard error, % *Ve* Percentage of variance explained, \*\*\*, \*\*, \*, *P*<0.001, 0.01, 0.05, respectively, ns: not significant. *P*-values for the explanatory variables refer to the *F*-tests



slightly but significantly in this experiment (Rose and Leuschner 2012). Various studies have demonstrated SRL increases in grassland plants in response to clipping (Jaramillo and Detling 1988; Thorne and Frank 2009), and intensive grazing was found to reduce root biomass (Matthew et al. 2000). We thus had expected higher SRL and SRA values in the more frequently mown plots in the year three of the experiment. Elevated carbon losses with harvested shoot biomass should promote strategies in which the soil is explored with less resource investment into root growth as it may be achieved by reductions in mean root diameter or tissue density. On the other hand, all six species are known to be moderately to highly tolerant to mowing as is expressed by the assignment of high scores (6 to 9) to these species in the 9-step mowing tolerance classification system of Dierschke and Briemle (2002) (*F. rubra* 9, *D. glomerata* and *R. repens*: 8, all other species 6–7). This system bases exclusively on the species' observed capacity for recovering above-ground biomass in frequently cut meadows in due time while belowground responses are ignored. The apparent insensitivity of the root system to mowing in our study seems to justify this system. However, we cannot exclude that the grassland plants in our experiment would respond to higher mowing frequencies (>3 cuts per year) since the C3 treatment simulates a management regime with only moderate and not high mowing intensity.

The second management factor, fertilization, was much more influential than mowing as we had assumed in hypothesis (2). Addition of  $180 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (plus adequate amounts of Ca, K and P) induced profound alterations in all five investigated root traits. In contrast, this treatment had no significant effect on standing root biomass after 2 years of fertilization (Rose and Leuschner 2012) indicating that root morphology and tissue N content are more sensitive to increased nutrient availability than carbon allocation patterns to aboveground and belowground organs in these grassland species. Probably the most consistent trait response was the SRA increase by up to 30 % in the fertilized plants as compared to the unfertilized ones which was observed in all species (partly as non-significant trend only) except for *V. chamaedrys*. This alteration was mainly a consequence of a reduction in root tissue mass density while a root diameter decrease played only a secondary role. This is indicated by the magnitude of the trait shifts and also by the

GLM analysis showing a stronger dependence of SRA on TMD than on RD in five species (exception: *A. capillaris*). A particularly large TMD reduction (by more than 30 %) was found in *R. acetosa*, followed by *A. capillaris* and *R. repens*. In contrast, mean root diameter was significantly reduced with NPK fertilization in only one species (*R. repens*). A somewhat different picture emerged for SRL and its dependence on TMD and RD. While SRL also increased in general with N addition revealing more or less congruent shifts with those of SRA, the GLMs revealed an apparently larger influence of root diameter than tissue density on SRL, even though the root diameter reduction with fertilization was small and mostly insignificant in the six species. Thus, increases in SRL were mostly driven by reductions in root diameter while SRA increases were mostly caused by reductions in tissue density.

The rather low plasticity of the root diameter together with the unchanged standing root biomass allows the conclusion that higher soil N availability did not lead to the development of a root system with much larger overall length but higher absorbing surface area. According to the supply pre-emption theories discussed by Craine (2005), Craine (2009) and Craine and Dybzinski (2013) a greater root length should be a competitive advantage in competition for nutrients. However, a higher absorbing area/high uptake capacity can facilitate a fast reduction of nutrients and thereby reduce the availability for possible competitors if nutrients are supplied in pulses and if root length densities are high. Absorbing surface area seemed to be more important under these conditions where competition for nutrients occurred on very small scales and in short time frames.

This pattern indicates that the six grassland species are able to modify SRL and SRA at least partly independently from each other through variation in either root diameter or mass per volume; in fact, RD and TMD were found to be non-correlated to each other in four of the six species (exceptions: *A. capillaris* and *D. glomerata*). If such a difference in the plasticity of root length and root surface area does exist, it might relate to the different functional roles of SRL and SRA: variation in root length relates to the volume of soil explored by roots (which would be important under patchy nutrient addition) while variation in surface area should have mainly an effect on the intensity of water and nutrient depletion per soil volume, which might be more significant under pulsed nutrient supply.

Our observation of increases in SRL, SRA and root N content with N addition matches with earlier reports on increased specific root length (Anderson 1988) and higher root N concentrations after N addition across different plant functional groups (Reich et al. 2003). Further, the reduced tissue mass density is in accordance with the observation that species growing on N-rich soils generally have lower root tissue densities (Ryser 1996); this increases N uptake per C investment in root growth (Eissenstat et al. 2000; Maire et al. 2009; Reich et al. 1998b; Richards 1984). Such a strategy should be advantageous when resource limitation is shifting from N to C limitation (Craine 2009) which is the case in intensively fertilized and frequently mown grasslands. Species with lower root tissue mass density should in general be more productive and thus more competitive in fertile environments (Casper and Jackson 1997), as is the case with plants with high SLA (Poorter and Remkes 1990). On the other hand, reduced tissue mass density should shorten root lifespan and thus increase the maintenance costs of the root system which may outweigh the benefit from reducing the amount of carbon invested in standing root biomass. However, shorter-lived roots may respond more rapidly to a pulse supply of nutrients as it occurs with fertilizer addition.

The six species increased their root N concentration upon NPK fertilization at varying degrees. The relatively slow-growing grass *A. capillaris* with the lowest N indicator value after Ellenberg et al. (1992) was the only species which did not show at least a tendency for increasing N in root mass; this might relate to the assumed relatively low N demand of this species.

## Conclusions

The results of our study demonstrate that the marked increase in grassland management intensity during the last 50 years must have resulted in profound changes in root morphological traits which mostly should have been caused by increased fertilizer application and not by the increased mowing frequency. Trait shifts were in most cases coherent among the species but the magnitude of change depended on the species and probably also on the systematic group (monocots vs. dicots). However, more species have to be examined before more general conclusions about systematic group differences can be drawn. Root morphological

traits may be used as valuable indicators of environmental change and increasing fertilization in grasslands but we need more information on root trait responses in additional species and under a broader set of conditions.

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