

## Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species

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• **Backgrounds and Aims** Leaf functional traits have been used as a basis to categorize plants across a range of resource-use specialization, from those that conserve available resources to those that exploit them. However, the extent to which the leaf functional traits used to define the resource-use strategies are related to root traits and are good indicators of the ability of the roots to take up nitrogen (N) are poorly known. This is an important question because interspecific differences in N uptake have been proposed as one mechanism by which species' coexistence may be determined. This study therefore investigated the relationships between functional traits and N uptake ability for grass species across a range of conservative to exploitative resource-use strategies.

• **Methods** Root uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and leaf and root functional traits were measured for eight grass species sampled at three grassland sites across Europe, in France, Austria and the UK. Species were grown in hydroponics to determine functional traits and kinetic uptake parameters ( $I_{\text{max}}$  and  $K_m$ ) under standardized conditions.

• **Key Results** Species with high specific leaf area (SLA) and shoot N content, and low leaf and root dry matter content (LDMC and RDMC, respectively), which are traits associated with the exploitative syndrome, had higher uptake and affinity for both N forms. No trade-off was observed in uptake between the two forms of N, and all species expressed a higher preference for  $\text{NH}_4^+$ .

• **Conclusions** The results support the use of leaf traits, and especially SLA and LDMC, as indicators of the N uptake ability across a broad range of grass species. The difficulties associated with assessing root properties are also highlighted, as root traits were only weakly correlated with leaf traits, and only RDMC and, to a lesser extent, root N content were related to leaf traits.

**Key words:** Ammonium, nitrate, plant functional traits, leaf traits, root traits, root nitrogen uptake, <sup>15</sup>N labelling, uptake rate, affinity, grasses, Poaceae, grassland ecology, resource-use strategy.

### INTRODUCTION

Plant resource-use strategies have received substantial attention over the last two decades as a means to better understand and predict the dynamics and functioning of ecosystems, particularly in the context of global change (Reich, 2014). It has been proposed that plant strategy theories (Grime, 1977; Tilman, 1990; Westoby *et al.*, 2002) can be underpinned by quantitative plant functional traits. In particular, some leaf traits, such as specific leaf area (SLA) or leaf dry matter content (LDMC), have been considered as indicators of a plant's capacity to acquire, use and recycle resources (Weiher *et al.*, 1999; Wright *et al.*, 2001). These traits are closely related to species photosynthetic efficiency and relative growth rate (Reich *et al.*, 1999; Garnier *et al.*, 2001; Wright *et al.*, 2004), as well as leaf life span (Reich *et al.*, 1999; Ryser and Urbas, 2000). The 'leaf economics spectrum' (Wright *et al.*, 2004) captures such

relationships by highlighting the link between SLA or leaf nitrogen concentration (LNC), and a spectrum running from an exploitative syndrome, with rapid turnover of nutrients in leaves resulting in fast growth, to a conservation syndrome associated with the fast conservation of nutrients in well-protected tissues and a resultant slow growth (Chapin, 1980; Poorter and De Jong, 1999; Wright *et al.*, 2004).

The existence of such a trade-off between acquisition and conservation of resources has also been suggested to occur for root traits (Roumet *et al.*, 2006; Fort *et al.*, 2013), and while relationships between root traits and functional processes linked to plant strategies are not as well documented as for leaves, several recent studies demonstrate the value of root traits as indicators of functions. Birouste *et al.* (2012), for example, found a relationship between root chemical composition and root potential decomposition rate for Mediterranean herbaceous species,

and Makita *et al.* (2012), studying the root systems of forest trees, identified relationships between root tissue density and specific root length (SRL), and coarse root respiration. Although specific environmental constraints may differentially affect above-ground and below-ground organs (Craine *et al.*, 2005; Tjoelker *et al.*, 2005; Roumet *et al.*, 2006; Chen *et al.*, 2013), integrated above- and below-ground syndromes can be linked, and collectively provide an explanation for resource-use strategies observed for the whole plant (Freschet *et al.*, 2010; Liu *et al.*, 2010; Fortunel *et al.*, 2012; Reich *et al.*, 2014).

Relationships between root N uptake and plant resource-use strategies as described by leaf traits have not yet been clearly established. Exploitative species are assumed to have high abilities to take up nutrients, as evidenced by the positive correlation between growth rate and N concentration in plants (Poorter *et al.*, 1990; Garnier, 1991). Some studies have strengthened this assumption by showing a link between the N absorption rate on the one hand, and SLA and/or leaf N concentration on the other (Osone *et al.*, 2008; James *et al.*, 2009; Leffler *et al.*, 2013). Root N uptake may depend on two components: root system morphology, which determines access to resources within soils, and physiological traits driving influx capacities. Root metabolic activity and nutrient uptake have indeed been related to root structural and chemical traits such as SRL or N concentration (Comas and Eissenstat, 2004; Tjoelker *et al.*, 2005; Bahn *et al.*, 2006; Maire *et al.*, 2009). It is now well established that the high affinity transport system transport (HATS) contributes mainly to the N uptake at low to moderate concentrations of external N and saturates at 0.2–0.5 mM (Kronzucker *et al.*, 2000; Min *et al.*, 2000), which makes it the more probable system used by plants growing in natural and semi-natural ecosystems limited by N (Bassirirad, 2000; Maire *et al.*, 2009). Thus, the uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  can be described by two kinetic parameters, the maximum uptake rate ( $V_{\text{max}}$ ) and the affinity constant or Michaelis constant ( $K_m$ ), to determine the ability and the efficiency of roots in absorbing  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . The Michaelis constant is commonly interpreted as the substrate ( $\text{NO}_3^-$  or  $\text{NH}_4^+$ ) concentration required to achieve 50 % of  $V_{\text{max}}$  and/or as the measure of HATS affinity for its substrate (low  $K_m$  values being equivalent to higher affinity). While some studies provide indirect support for the idea that slow-growing plants occurring in nutrient-poor environments have a higher affinity of transport systems for N (lower  $K_m$ ) (Garnier *et al.*, 1989; Muller and Garnier, 1990), others have not found any evidence to support such a relationship (Freijesen and Otten, 1984; Bloom, 1985; Oscarson *et al.*, 1989). In addition, due to a lack of comparative studies characterizing  $K_m$  under standardized conditions for a range of species, the relationship between  $K_m$  for N and species resource-use strategies (Lambers and Poorter, 1992) is still poorly understood. Such studies would also be needed to determine whether higher affinity (low  $K_m$  values), which allows species to exploit N resources at lower soil concentrations, is a characteristic of slow-growing species or a characteristic of fast-growing species.

The overall goal of our study was to test for relationships between N uptake ability and functional traits of a broad range of grass species representing contrasting resource-use strategies. To do this, we grew plants taken from three grassland sites across Europe under standardized conditions, and measured their leaf and root functional traits, and estimated uptake

parameters (maximum uptake rate,  $I_{\text{max}}$ ; and Michaelis–Menten affinity constant,  $K_m$ ) for the main inorganic N forms available for plant nutrition, ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ). Specifically, we addressed the following questions. (1) What are the relationships between root functional traits and the resource-use strategy of species as described by leaf traits? (2) Are exploitative species more efficient in taking up  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (higher  $I_{\text{max}}$ ) compared with conservative species? (3) Do conservative species, which generally grow in N-poor environments, have higher affinity (lower  $K_m$ ) for both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  than exploitative species? We hypothesized that exploitative species are more efficient in acquiring N when its availability is high (high uptake rate), and conservative species are more efficient in acquiring N when its availability is low (low uptake rate but higher affinity).

## MATERIALS AND METHODS

### *Plant species and cultures*

Eight grass species were chosen to represent common species at three European grassland sites encompassing a large range of functional diversity (see Grigulis *et al.*, 2013 for detailed site descriptions). Briefly, the three sites represented different levels of overall grassland management intensity, and were dominated by different species ranging from conservative to exploitative strategies. *Briza media*, *Bromus erectus*, *Dactylis glomerata*, *Festuca paniculata* and *Sesleria caerulea* were sampled at the French site located in the French Alps, near the Lautaret Pass (45°25'1"N, 6°22'43'5"E, elevation 1700–2000 m). *Dactylis glomerata* and *Nardus stricta* were sampled at the Austrian site located in the Stubai Alps (47°7'46'10"N, 11°18'21'60"E, elevation: 1800–2000 m). *Anthoxanthum odoratum*, *Dactylis glomerata* and *Lolium perenne* were sampled at the UK site located in the Yorkshire Dales (UK) (54°18'31'8"N, 2°4'53'8"E, elevation: 200 m). *Dactylis glomerata* was collected across all sites as a control to ensure that observed differences between species would be linked to differences in plant strategies and not to the origins of plants.

For each species, a few individuals (3–5) were sampled in the field and vegetatively multiplied on floating perlite at the University of Caen in a greenhouse (16 h day 20 °C/8 h night 16 °C) with additional light provided by sodium lamps (400 W Philips SON T-PIA Agro, providing 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation). Plants were supplied with a nutritive solution renewed every week that contained 1 mM  $\text{NH}_4\text{NO}_3$ , 0.18 mM  $\text{CaCO}_3$ , 0.4 mM  $\text{KH}_2\text{PO}_4$ , 0.15 mM  $\text{K}_2\text{HPO}_4$ , 3 mM  $\text{CaCl}_2$ , 0.2 mM EDTA, 2NaFe·H<sub>2</sub>O, 14  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 5  $\mu\text{M}$   $\text{MnSO}_4\cdot\text{H}_2\text{O}$ , 3  $\mu\text{M}$   $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ , 0.7  $\mu\text{M}$   $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ , 0.7  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  and 0.1  $\mu\text{M}$   $\text{CaCl}_2$ . During the multiplication step, we regularly cut (5 cm) the aerial and root parts in order to favour the production of new tissues. This multiplication step ran for 2–3 months depending on the growth of species, and was designed to standardize the growing conditions of plants and species coming from different sites as well as to provide enough replicates to estimate N uptake (approx. 100 tillers per species). Before the labelling experiment, several tillers used as replicates were grown in hydroponic culture with the same solution renewed every 3 d to prevent any nutrient limitation, and without cutting. This last period of growth ran until individuals

reached the stage of 3–4 tillers (usually between 2 and 3 weeks) in order to ensure a similar phenological status for all species. We assumed that the larger number of replicate tillers randomly used for the different analyses represented the genetic variation of the mother plants sampled in the field.

#### Nitrogen labelling and functional traits

$^{15}\text{N}$  labelling was used to determine kinetic parameters of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake by the different plants studied. Following Glass (2000), kinetic parameter measurements were applied to determine incoming  $^{15}\text{N}$  influx (rather than net uptake, which is the resultant of influx and efflux). For this reason, the duration of  $^{15}\text{N}$  labelling did not exceed 5 min. The plant roots were washed twice for 1 min with a solution of calcium sulphate ( $\text{CaSO}_4$ ; 1 mM), then transferred over 5 min to a nutritive solution containing  $^{15}\text{NO}_3^-$  ( $\text{K}^{15}\text{NO}_3$ ;  $^{15}\text{N}$  excess = 99 %) or  $^{15}\text{NH}_4^+$  [ $(^{15}\text{NH}_4)_2\text{SO}_4$ ;  $^{15}\text{N}$  excess = 99 %], then washed again twice for 1 min with a 1 mM  $\text{CaSO}_4$  solution maintained at 4 °C to stop any energetic processes. We labelled the whole root plant system across a range of concentrations (<1 mM) relevant to estimate HATS activity and kinetic N uptake parameters of plants. For each species and N form, kinetic parameters of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake were determined from three sets of measurement. Each set of measurements corresponds to one N kinetic uptake, obtained by cultivating several replicates obtained after the multiplication phase for 5 min at the different labelled N concentrations along an increasing gradient (20–30–40–50–75–100–150–250–500–750  $\mu\text{M}$ ), providing at least 30 ‘replicates’ along the gradient to estimate the N uptake kinetics for each N form (see below). During the labelling, roots were attached to the shoot; therefore, our measurements represent the whole-plant N uptake. At the end of the labelling, shoots and roots of all replicates were weighed and analysed separately.

The total N amount and  $^{15}\text{N}$  excess were determined by analysing samples after oven-drying at 60 °C during 72 h and grinding to a fine powder, with a continuous flow isotope mass spectrometer (Isoprime, GV Instruments, Manchester, UK) linked to a C/N/S analyser (EA3000, Euro Vector, Milan, Italy). The total N ( $N_{\text{tot}}$ ) content of a given replicate ‘i’ was calculated as:  $N_{\text{tot}i} = (\%N_i \times \text{DM}_i) / 100$ . The natural  $^{15}\text{N}$  abundance ( $0.3663 \pm 0.0004$  %) of atmospheric  $\text{N}_2$  was used as a reference for  $^{15}\text{N}$  analysis. Nitrogen derived from current N uptake ( $Q^{15}\text{N}$ ) in a given replicate was calculated as:  $Q^{15}\text{N} = (N_{\text{tot}i} \times E_i) / E_s$ , where  $E_i$  (%) is the atom  $^{15}\text{N}$  excess and  $E_s$  is the nutrient solution atom  $^{15}\text{N}$  excess. Then,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  influx was expressed as  $\mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  dry root or  $\mu\text{mol NH}_4^+ \text{ h}^{-1} \text{ g}^{-1}$  dry root, respectively (Fig. 1).

We measured leaf and root functional traits using standardized protocols (Cornelissen *et al.*, 2003) for 6–10 replicates of each species (see Table 1) similar to those used for N uptake estimation. Because plants were grown under optimal conditions in hydropony, we did not rehydrate leaves before measurement, but kept leaves in ice until measurement, <2 h after harvest. Leaf area (LiCor 3100), fresh mass and dry mass (after drying at 60 °C during 48 h) of the last mature leaf were measured to assess SLA and LDMC. Fresh mass and dry mass of roots as well as root length (Winrhizo<sup>®</sup> software) were measured to

assess root dry matter content (RDMC) and SRL. The allocation of biomass to shoots or roots was evaluated by the ratio of their dry masses, i.e. the shoot:root ratio (SRR).

#### Kinetic uptake parameter estimation

Kinetic uptake parameters ( $I_{\text{max}}$ , maximum influx;  $K_m$ , Michaelis constant) for HATS were determined to compare the efficiency of species to take up  $\text{NO}_3^-$  and  $\text{NH}_4^+$  from the solution. Following Engels *et al.* (2000), Hanes’s relationship (Michaelis–Menten transformed equation) was used to fit the dependency of the influx rate on the substrate concentration ( $C$ ;  $\mu\text{M}$ ) allowing the direct calculation of the maximal influx rate of  $\text{NO}_3^-$  or  $\text{NH}_4^+$  from the slope of the linear curve:  $1/\text{influx} = K_m/I_{\text{max}} \times C + 1/I_{\text{max}}$ .

The specific influx capacity was calculated as the maximal influx rate per unit of root dry mass. Substrate affinity ( $K_m$ ) of the HATS was calculated from the intercept divided by the slope of the linear curve.  $K_m$  translates the efficiency to take up N inorganic compounds at low concentrations: a low  $K_m$  value characterizes a high affinity of HATS for  $\text{NO}_3^-$  or  $\text{NH}_4^+$ .

#### Data analysis

Relationships between functional traits were assessed using Pearson correlation coefficients. A principal component analysis (PCA) was used to explore the distribution of species relative to their functional traits and uptake parameters for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Species differences for uptake parameters were tested using a one-way analysis of variance (ANOVA), followed by a Tukey post-hoc test. All statistical analyses were performed with the statistical software R 2.14 (R Development Core Team, 2012), and the PCA analysis was carried out with the *ade4* package (Chessel *et al.*, 2004).

## RESULTS

#### Leaf and root trait syndromes

The studied species encompassed a large spectrum of variability for functional traits, with SLA ranging from 4 to 27  $\text{mm}^2 \text{ g}^{-1}$ , LDMC from 204 to 436  $\text{mg g}^{-1}$  and  $N_{\text{shoot}}$  from 2.5 to 4.3  $\text{mg g}^{-1}$  (Table 1). Variations for LDMC, SLA and  $N_{\text{shoot}}$  in our experiment represented 35, 28 and 27 %, respectively, of the variation reported for a wider range of species and life forms in Cornelissen *et al.* (2003). In the PCA (Fig. 2), species spanned mainly along the first axis (42.29 % of total inertia) from a conservative syndrome with high values of LDMC and RDMC associated with low values of SLA and  $N_{\text{shoot}}$  for species such as *N. stricta*, *S. caerulea* and *F. paniculata*, to an exploitative syndrome with high values of SLA and  $N_{\text{shoot}}$  associated with kinetic low values of LDMC and RDMC for species such as *D. glomerata* or *A. odoratum*. The second axis (19.94 % of total inertia) was related to variations in SRR and SRL. Consequently, SRR and SRL were orthogonal to the first axis and thus independent of species strategies. Both SRR and SRL are indeed not correlated with the main contributing traits to the first axis ( $N_{\text{shoot}}$ , SLA, LDMC and RDMC). Among traits, the strongest correlation was the negative one between



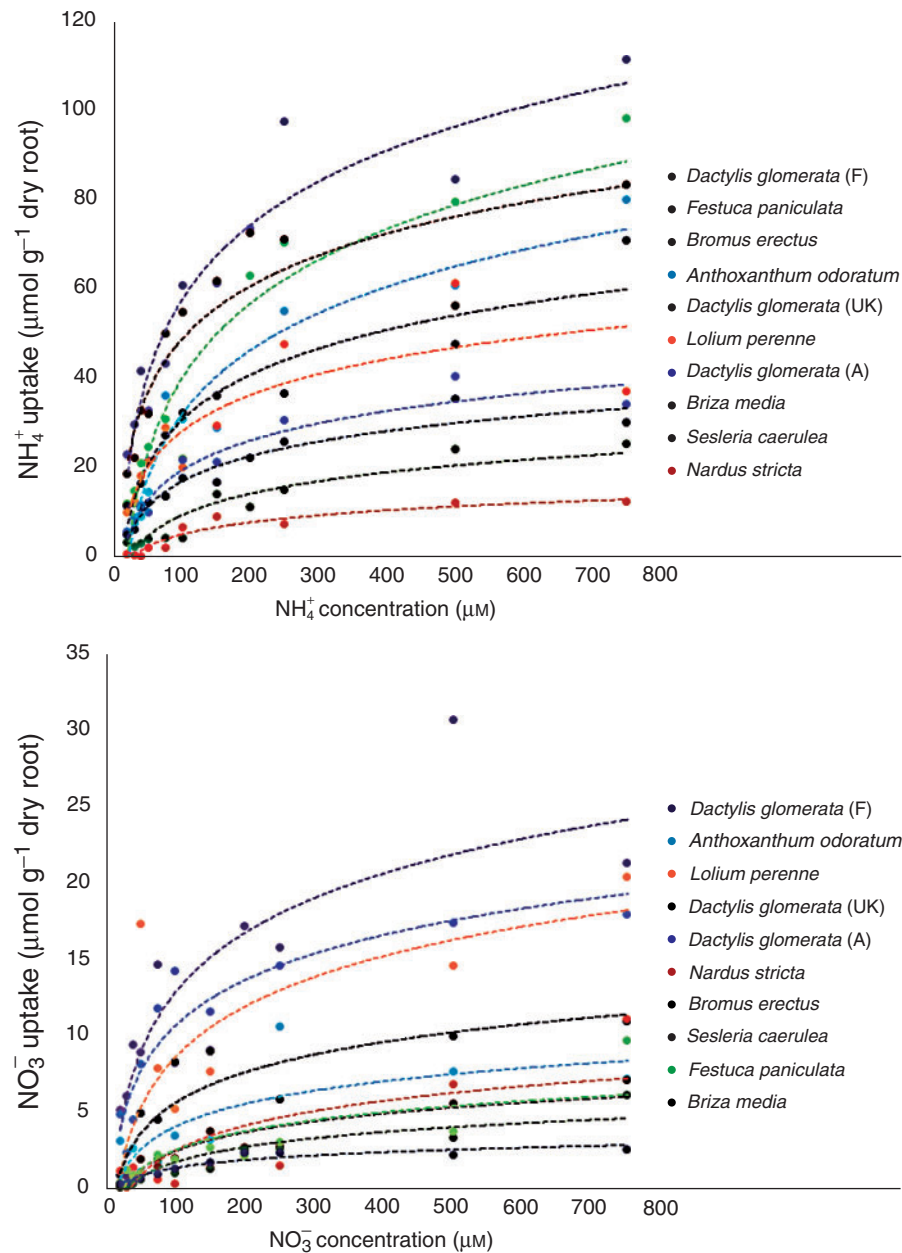


Fig. 1. Nitrogen uptake rates of  $\text{NH}_4^+$  (top) and  $\text{NO}_3^-$  (bottom) at the different substrate concentrations. Species names in the key are ordered following the position of the regression line at the end of the N concentration axis to simplify the reading of the figure. However, the order was not necessarily the same as the ranking based on  $V_{\max}$  values presented in Fig. 3, which were calculated using all the points and not only the mean values per concentration presented here.

SLA and LDMC ( $r = -0.76$ ,  $P < 0.001$ ), two main contributors to the leaf economics spectrum. Those leaf traits were correlated with  $N_{\text{shoot}}$  (with SLA,  $r = 0.60$ ,  $P < 0.001$ , and with LDMC,  $r = -0.58$ ,  $P < 0.001$ ). Among root traits, only RDMC was well correlated to the leaf economics spectrum (with SLA,  $r = -0.46$ ,  $P < 0.001$ ; with LDMC,  $r = 0.30$ ,  $P < 0.01$ ; and with  $N_{\text{shoot}}$ ,  $r = -0.57$ ,  $P < 0.001$ ). Of SRL and  $N_{\text{root}}$ , which were chosen to characterize economic aspects of the root system, the second was the more relevant trait when studying plant strategies, because of high correlation with SRL ( $r = 0.30$ ,  $P < 0.01$ ) and with two of the traits contributing to the first axis (with  $N_{\text{shoot}}$ ,  $r = 0.70$ ,  $P < 0.001$ ; and with RDMC,  $r = 0.38$ ,

$P < 0.001$ ). The SRR, which reflects the partitioning of biomass to above- vs. below-ground plant parts, was not correlated with any of the other traits.

#### Kinetic parameters of $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake

The  $I_{\max}$  values expressed per mass unit of root mass and unit of root length were strongly correlated ( $r = 0.86$ ,  $P < 0.001$ ); therefore, we only present  $I_{\max}$  expressed on a mass basis. The  $I_{\max}$  for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  differed between species ( $F$ -ratio = 6.02 and 3.90,  $P$ -value  $< 0.001$  and  $< 0.01$ ,

TABLE 1. Species effect on the different traits

Species	SLA (mm <sup>2</sup> mg <sup>-1</sup> )	LDMC (mg g <sup>-1</sup> )	N <sub>shoot</sub> (%)	SRL (m g <sup>-1</sup> )	RDMC (mg g <sup>-1</sup> )	N <sub>root</sub> (%)	SRR
<i>Anthoxanthum odoratum</i> (10)	21.93 <sup>ab</sup> (1.91)	297.49 <sup>bc</sup> (23.11)	4.19 <sup>a</sup> (0.17)	79.48 <sup>ab</sup> (9.71)	77.93 <sup>d</sup> (2.99)	4.40 <sup>ab</sup> (0.16)	10.44 <sup>ab</sup> (0.96)
<i>Briza media</i> (6)	11.05 <sup>c</sup> (1.09)	326.80 <sup>ab</sup> (22.18)	3.37 <sup>bc</sup> (0.16)	116.54 <sup>ab</sup> (12.31)	118.47 <sup>abc</sup> (2.65)	3.55 <sup>bc</sup> (0.15)	2.88 <sup>c</sup> (0.32)
<i>Bromus erectus</i> (6)	14.37 <sup>c</sup> (1.36)	262.10 <sup>b</sup> (15.45)	4.27 <sup>a</sup> (0.30)	104.42 <sup>ab</sup> (13.91)	133.74 <sup>ab</sup> (7.23)	4.74 <sup>a</sup> (0.49)	2.62 <sup>c</sup> (0.21)
<i>Dactylis glomerata</i> (A) (10)	20.60 <sup>b</sup> (1.65)	227.17 <sup>c</sup> (7.52)	4.09 <sup>a</sup> (0.13)	47.98 <sup>b</sup> (7.44)	131.47 <sup>ab</sup> (12.31)	2.86 <sup>c</sup> (0.17)	3.38 <sup>c</sup> (0.27)
<i>Dactylis glomerata</i> (F) (6)	26.88 <sup>a</sup> (1.69)	204 <sup>c</sup> (12.62)	3.80 <sup>ab</sup> (0.2)	105.46 <sup>ab</sup> (18.26)	119.19 <sup>ab</sup> (9.50)	2.93 <sup>c</sup> (0.17)	4.00 <sup>bc</sup> (0.35)
<i>Dactylis glomerata</i> (UK) (10)	24.74 <sup>ab</sup> (1.09)	215.89 <sup>c</sup> (8.36)	4.14 <sup>a</sup> (0.08)	56.49 <sup>b</sup> (4.81)	71.56 <sup>d</sup> (3.49)	2.98 <sup>c</sup> (0.15)	3.63 <sup>bc</sup> (0.27)
<i>Festuca paniculata</i> (6)	8.81 <sup>cd</sup> (1.35)	321.25 <sup>b</sup> (32.65)	2.82 <sup>cd</sup> (0.15)	150.26 <sup>a</sup> (54.55)	159.63 <sup>a</sup> (21.42)	3.23 <sup>c</sup> (0.22)	12.14 <sup>a</sup> (2.36)
<i>Lolium perenne</i> (10)	15.41 <sup>c</sup> (0.64)	275.10 <sup>bc</sup> (10.68)	4.20 <sup>a</sup> (0.05)	98.23 <sup>a</sup> (14.32)	90.50 <sup>cd</sup> (2.38)	4.23 <sup>ab</sup> (0.08)	4.26 <sup>bc</sup> (0.16)
<i>Nardus stricta</i> (10)	4.18 <sup>d</sup> (0.17)	435.97 <sup>a</sup> (35.54)	2.53 <sup>d</sup> (0.14)	41.88 <sup>b</sup> (5.57)	151.82 <sup>ab</sup> (20.50)	1.89 <sup>d</sup> (0.21)	1.64 <sup>c</sup> (0.16)
<i>Sesleria caerulea</i> (6)	9.55 <sup>cd</sup> (1.22)	358.40 <sup>ab</sup> (18.65)	3.23 <sup>bc</sup> (0.11)	62.29 <sup>b</sup> (21.21)	115.04 <sup>bc</sup> (12.67)	2.74 <sup>cd</sup> (0.23)	7.06 <sup>b</sup> (1.49)
F-ratio	36.88	14.01	22.25	4.48	15.95	21.99	22.87

Mean and standard error in parentheses are given for functional traits for each species.

The number of replicates is indicated for each species in parentheses.

The *F*-ratio (*P*-value < 0.001 for all traits) after an ANOVA testing the differences between species for each trait is indicated (*n* = 80).

See Fig. 2 and the text for a full description of the trait acronyms.

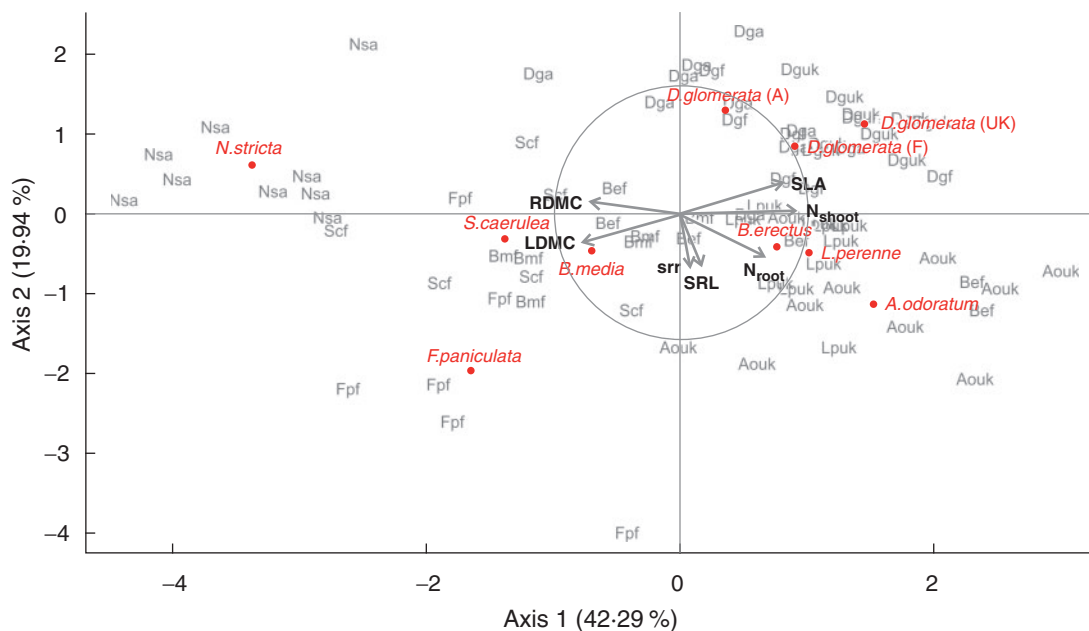


FIG. 2. Principal component analysis (PCA) based on functional traits measured for ten replicates (in grey) of species used in the study (multivariate mean indicated in red). SRL, specific root length; SRR, shoot:root ratio; SLA, specific leaf area; LDMC, leaf dry matter content; N<sub>shoot</sub> and N<sub>root</sub>, nitrogen concentration in the shoot and root, respectively. For *D. glomerata*, the site of origin is indicated in parentheses. Total variance explained by the two first axes: 62.93 %. Loading scores for the two first axes of the PCA for leaf and root traits are given in Supplementary Data Table S2.

respectively, Fig. 3A, B). The  $I_{\max}$  values for all species were higher for  $\text{NH}_4^+$  (26–110  $\mu\text{mol h}^{-1} \text{g}^{-1}$ ) than for  $\text{NO}_3^-$  (3–27  $\mu\text{mol h}^{-1} \text{g}^{-1}$ ).

The  $K_m$  reflects the ability of species to exploit N resources when their availability is very low, lower  $K_m$  values indicating higher affinity for the substrate. The  $K_m$  for both N forms differed between species (*F*-ratio = 6.53 and 5.8, *P*-value < 0.001 for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively, Fig. 3C, D). *Dactylis glomerata* (from all sites) had low  $K_m$  values for both N forms, while *N. stricta*, *A. odoratum* and *S. caerulea* had high values for both forms (Table 1).  $K_m$  values for both N forms were correlated ( $r = 0.70$ ,  $P < 0.05$ ).

More exploitative species such as *D. glomerata* had a higher  $I_{\max}$  and lower  $K_m$  (higher affinity) for both N forms compared

with more conservative species such as *N. stricta* and *S. caerulea*. The hypothesis of a negative correlation between  $K_m$  and  $I_{\max}$  was tested, but no relationship was found for  $\text{NH}_4^+$  or for  $\text{NO}_3^-$  ( $\text{NH}_4^+$ ,  $r = -0.12$  n.s.;  $\text{NO}_3^-$ ,  $r = -0.16$  n.s.). Indeed, although this pattern held for some species, other species such as *A. odoratum* or *B. media* had high or low values, respectively, for both uptake parameters studied.

In order to investigate the relationships between uptake ability and resource use-strategies, as defined by functional traits, we analysed the relationship between kinetic parameters and species co-ordinates in the first axis of the PCA, which was identified as describing a resource-use strategy continuum from conservative to exploitative species. For both N forms,  $I_{\max}$  was positively correlated with the PCA first axis, meaning higher N

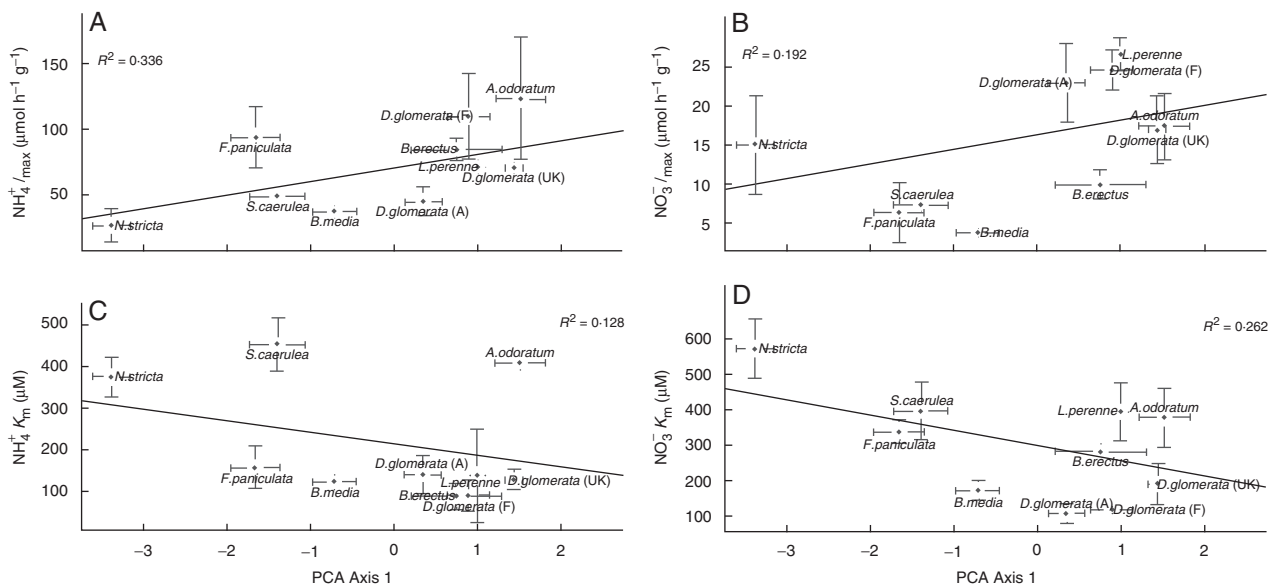


Fig. 3. Relationships between uptake parameters. Maximum uptake rate per gram of dry root mass for (A)  $\text{NH}_4^+$  and (B)  $\text{NO}_3^-$ , affinity for (C)  $\text{NH}_4^+$  and (D)  $\text{NO}_3^-$  and the co-ordinates of individuals along the first axis of the PCA.  $R^2$  values for significant linear regression between variables ( $P$ -value  $< 0.05$ ) are indicated.

uptake for exploitative species (Fig. 3).  $\text{NH}_4^+$  and  $\text{NO}_3^- K_m$  values were negatively correlated with the PCA axis, meaning that the exploitative species had higher affinity for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . All these relationships were also significant when the site of origin was taken into account (Supplementary Data Table S1). The specific examination of relationships between kinetic parameters and individual plant traits LDMC and SLA revealed that they appeared to be the more closely related, particularly with  $K_m$  (Table 2).

## DISCUSSION

### Relationships between root and leaf functional traits

The set of species in our study covered a representative range for herbaceous species for leaf functional traits involved in the leaf economics spectrum, spanning from more conservative to more exploitative (Cornelissen et al., 2003; Diaz et al., 2004; Wright et al., 2004). Conservative species such as *S. caerulea* or *N. stricta* were characterized by low SLA and  $N_{\text{shoot}}$  and high LDMC, indicating a low rate of tissue turnover adapted to nutrient-poor environments (Reich et al., 1992; Wilson et al., 1999). At the other extreme of the range, high SLA and low LDMC observed for *D. glomerata*, were consistent with an exploitative strategy typical of nutrient-rich sites (Garnier et al., 2001; Hodgson et al., 2011). Functional root traits are increasingly being included in the resource economics spectrum (Mommer and Weemstra, 2012), and our results give some support to this. Functional root traits such as RDMC and  $N_{\text{root}}$  contributed significantly to the axis of specialization of species from conservation to exploitation. However, except for RDMC, root traits in our study were poorly correlated with leaf functional traits. Despite the lack of relationships between leaf and root traits, parallel contributions of leaf and root traits to the plant economics spectrum were apparent for the eight investigated grass species, as well as in other studies reporting similar functional

syndromes despite various degrees of relationships between functional traits (Tjoelker et al., 2005; Roumet et al., 2006; Freschet et al., 2010; Fort et al., 2013).

Analogous functional traits for leaves and roots have already been related to similar plant features above- and below-ground, such as dry matter content to tissue longevity (Withington et al., 2006; McCormack et al., 2012), or N content to respiration rate (Reich et al., 1998; Tjoelker et al., 2005). However, we observed a second axis of differentiation between species involving variations in SRL and SRR, two traits that were poorly correlated with leaf traits or with the first axis. High SRL and low SRR are often associated with higher N uptake for exploitative species (Eissenstat, 1992; Reich et al., 1998; Ryser, 1998). However, the generality of the importance of those root traits in the exploitative strategy is still unknown since either positive relationships or absence of relationships have been reported (Craine and Lee, 2003; Tjoelker et al., 2005, respectively) between SRL and SLA, the analogous leaf trait for light capture (Wright et al., 2004). Different traits involved in the capture of different resources could be hypothesized to diverge if access to the resources differ. For example, leaf and root traits are more likely to co-vary if species compete both for light and for soil nutrients (e.g. Craine and Lee, 2003) than if access to the resources is more unrelated due to different environmental conditions (Chen et al., 2013; Freschet et al., 2013). Since root traits strongly depend on abiotic soil conditions (Robinson and Rorinson, 1988; Hodge, 2004), hydroponic conditions such as in our study, removing the physical constraints on root growth (Craine and Lee, 2003; Freschet et al., 2013), are a possible explanation for the lack of relationship between SLA and SRL. Also, having easy access to large amounts of nutrients could explain why N concentrations were observed to be well correlated between leaves and roots, while a trait related to the morphology such as SRL may be more important for access to the resource, which is less a problem for plants in hydropony. Overall, in spite of a low degree of correlation with

TABLE 2. Pearson correlation between the species mean ( $n = 10$ ) for functional traits measured for leaves (SLA, LDMC,  $N_{shoot}$ ), roots (SRL, RDMC,  $N_{root}$ ) and N uptake parameters ( $I_{max}$  and  $K_m$  for  $NH_4^+$  and  $NO_3^-$ )

	SLA	LDMC	$N_{shoot}$	SRL	RDMC	$N_{root}$	srr	$NH_4^+ I_{max}$	$NO_3^- I_{max}$	$NH_4^+ K_m$	$NO_3^- K_m$
SLA	–										
LDMC	<b>–0.89</b>	–									
$N_{shoot}$	<b>0.77</b>	<b>–0.80</b>	–								
SRL	–0.10	–0.12	–0.08	–							
RDMC	<i>–0.63</i>	0.43	<b>–0.68</b>	0.26	–						
$N_{root}$	0.24	–0.35	<b>0.67</b>	0.47	–0.37	–					
SRR	–0.03	0.09	–0.15	0.44	–0.02	0.21	–				
$NH_4^+ I_{max}$	<i>0.58</i>	–0.49	0.44	0.46	–0.32	<i>0.55</i>	<i>0.58</i>	–			
$NO_3^- I_{max}$	<i>0.62</i>	–0.52	0.52	–0.34	–0.38	0.01	–0.25	0.25	–		
$NH_4^+ K_m$	–0.38	<b>0.68</b>	–0.39	–0.47	–0.07	–0.27	0.34	–0.12	–0.20	–	
$NO_3^- K_m$	<b>–0.68</b>	<b>0.82</b>	–0.50	–0.18	0.17	–0.11	0.19	–0.17	–0.16	<b>0.70</b>	–

$P < 0.05$  is indicated by bold;  $P < 0.1$  is indicated by italics. Other values are not significant.

leaf traits, our results highlighted that root traits are likely to contribute to the plant economics spectrum (Craine *et al.*, 2005; Tjoelker *et al.*, 2005; Roumet *et al.*, 2006; Freschet *et al.*, 2010; Reich, 2014).

#### Relationships between functional traits and N uptake

The exploitative strategy involves morphological and physiological traits such as high SLA and photosynthetic N-use efficiency (Poorter *et al.*, 1990; Reich *et al.*, 1999; Wright *et al.*, 2001), which should be associated with higher ability to capture nutrients in response to higher N soil availability. While N acquisition has been demonstrated to be an important feature associated with this response (Osone *et al.*, 2008; James *et al.*, 2009), very few studies have investigated the relationships between functional traits involved in the resource economics spectrum and root N uptake, especially under controlled conditions that remove confounding effects of temperature, pH or N availability on N uptake (Louahlia *et al.*, 1999; Jumpponen *et al.*, 2002; Warren, 2009). Our results highlighted higher  $NO_3^-$  and  $NH_4^+$  uptake ability for more exploitative strategy species (Maire *et al.*, 2009), as well as higher affinity for both N forms. Surprisingly, the best predictors of N uptake and affinity for  $NO_3^-$  and  $NH_4^+$  were two leaf structural traits, SLA and LDMC, rather than analogous root traits describing access to soil resources, such as SRL. Also, root or leaf N concentrations were poorly related to N uptake, supporting the idea that plant N concentration does not provide a direct indicator of the ability of species to take up N, as is commonly assumed, since other plant traits also influence N uptake (James, 2008). Although leaf and root traits were only weakly correlated in our experiment, leaf functional traits still appeared to be good functional markers of plant functioning (Garnier *et al.*, 2004), with our results indicating higher uptake ability for species with functional traits indicative of a more exploitative strategy.

Our results also have intriguing implications for mechanisms of community assembly. Niche partitioning for different N forms has been proposed as a mechanism allowing species coexistence (Tilman, 1994; Miller and Bowman, 2002; Harrison *et al.*, 2007). While there is some field evidence for preferential use of different N forms by coexisting plants (Ashton *et al.*, 2010; but see Harrison *et al.*, 2007), and that such specialization

could be expressed as a trade-off in the uptake of  $NO_3^-$  and  $NH_4^+$  between exploitative and conservative species, respectively (Maire *et al.*, 2009), our results do not support such findings. The uptake rates for  $NO_3^-$  and  $NH_4^+$  were not significantly correlated, and we even observed a positive relationship between affinities for the two N forms. We nevertheless acknowledge that our study was not specifically designed to deal with how N uptake strategies contribute to species coexistence. Furthermore, numerous field studies highlighted that N uptake of species is strongly influenced by soil N availability (Houlton *et al.*, 2007; Stahl *et al.*, 2011; Wang and Macko, 2011), which is likely to differ between habitats. However, using different species in similar conditions, especially in terms of N availability, we demonstrated that species N uptake ability was related to their functional traits, and consequently to their resource-use strategy. Further, we observed higher affinity for  $NH_4^+$  and  $NO_3^-$  for species with a more exploitative strategy, a result that contradicts the assumption that high affinity could be an attribute of species occurring under low nutrient availability (Lambers and Poorter, 1992; Näsholm *et al.*, 2000) such as a conservative species. However, more investigations are needed to draw firm conclusions about these relationships. Indeed, while our experiment demonstrated that species with a more exploitative strategy had higher N uptake ability and affinity in near optimal conditions (hydropony), there is increasing evidence that both N preferences and N uptake ability of species depending on N availability in natural conditions are important determinants of species distributions (Maire *et al.*, 2009; Ashton *et al.*, 2010; Andersen and Turner, 2013). Therefore, future studies are needed to test whether the distinct patterns of N uptake between exploitative and conservative species observed in our study for a large set of species under controlled conditions exist in the field across gradients of soil fertility.

#### Conclusions

Using eight grass species covering a broad range of leaf trait values under controlled conditions, we highlighted that not only leaf functional traits but also a root trait, RDMC, contribute to the resource economics spectrum distinguishing conservative and exploitative strategies. Moreover, we observed higher root N uptake rates and affinity for exploitative species, suggesting



greater ability to take up N from soil under a large range of N availability levels. Future studies should test whether the distinct patterns of N uptake between exploitative and conservative species observed under controlled conditions is also confirmed across gradients of soil fertility under natural conditions.

#### SUPPLEMENTARY DATA

Supplementary data are available online at [www.aob.oxfordjournals.org](http://www.aob.oxfordjournals.org) and consist of the following. **Table S1:** linear mixed model with  $I_{\max}$  and  $K_m$  for ammonium and nitrate as response variables, and the first axis of the PCA and site as explanatory variables. **Table S2:** loading scores along the two first axes of the PCA for leaf and root traits.

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