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

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Received: 27 May 2014 Returned for Revision: 26 August 2014 Accepted: 24 September 2014

• 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 NH4+ and NO3−, 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 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 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 NH4+.

• 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,15N 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 (Weih 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 Urban, 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 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; Freijsen and Otten, 1984; Bloom, 1985; Oscarson et al., 1989).

Thus, the uptake of NO₃⁻ and NH₄⁺ can be described by two kinetic parameters, the maximum uptake rate (Vmax) and the affinity constant or Michaelis constant (Km), to determine the ability and the efficiency of roots in absorbing NO₃⁻ and NH₄⁺. The Michaelis constant is commonly interpreted as the substrate (NO₃⁻ or NH₄⁺) concentration required to achieve 50 % of Vmax and/or as the measure of HATS affinity for its substrate (low Km 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 Km values) (Garnier et al., 1989; Muller and Garnier, 1990), others have not found any evidence to support such a relationship (Freijsen and Otten, 1984; Bloom, 1985; Oscarson et al., 1989).

In addition, due to a lack of comparative studies characterizing Km under standardized conditions for a range of species, the relationship between Km 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 Km 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, Imax; and Michaelis–Menten affinity constant, Km) for the main inorganic N forms available for plant nutrition, ammonium (NH₄⁺) and nitrate (NO₃⁻). 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 NO₃⁻ and NH₄⁺ (higher Imax) compared with conservative species? (3) Do conservative species, which generally grow in N-poor environments, have higher affinity (lower Km) for both NO₃⁻ and NH₄⁺ 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°45′3″8″, 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 μmol m⁻² s⁻¹ photosynthetically active radiation). Plants were supplied with a nutritional solution renewed every week that contained 1 mM NH₄NO₃, 0.18 mM CaCO₃, 0.4 mM KH₂PO₄, 0.15 mM K₂HPO₄, 3 mM CaCl₂, 0.2 mM EDTA, 2NaFe-EDTA, 14 μM H₂BO₃, 5 μM MnSO₄·H₂O, 3 μM ZnSO₄·7H₂O, 0.7 μM CuSO₄·5H₂O, 0.7 μM (NH₄)₂MoO₄·2H₂O and 0.1 μM CaCl₂. 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

15N labelling was used to determine kinetic parameters of NO₃ and NH₄⁺ uptake by the different plants studied. Following Glass (2000), kinetic parameter measurements were applied to determine incoming 15N influx (rather than net uptake, which is the resultant of influx and efflux). For this reason, the duration of 15N labelling did not exceed 5 min. The plant roots were washed twice for 1 min with a solution of calcium sulphate (CaSO₄; 1 mM), then transferred over 5 min to a nutritive solution containing 15NO₃ (K15NO₃; 15N excess = 99 %) or 15NH₄ [(15NH₄)₂SO₄; 15N excess = 99 %], then washed again twice for 1 min with a 1 mM CaSO₄ 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 NO₃ and NH₄⁺ 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 μM), 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 15N 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 (Isoprobe; GV Instruments, Manchester, UK) linked to a C/N/S analyser (EA3000, Euro Vector, Milan, Italy). The total N (Ntot) content of a given replicate ‘i’ was calculated as: Ntot = (%N × DM)/100. The natural 15N abundance (0.3663 ± 0.0004 %) of atmospheric N₂ was used as a reference for 15N analysis. Nitrogen derived from current N uptake (Q15N) in a given replicate was calculated as: Q15N = (Ntot × Ei)/Eo, where Ei ( %) is the 15N excess and Eo is the nutrient solution atom 15N excess. Then, NO₃ and NH₄⁺ influx was expressed as μmol NO₃ h⁻¹ g⁻¹ dry root or μmol NH₄⁺ h⁻¹ 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® 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 (Iₘax, maximum influx; Kᵅ, Michaelis constant) for HATS were determined to compare the efficiency of species to take up NO₃ and NH₄⁺ 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; μM) allowing the direct calculation of the maximal influx rate of NO₃ or NH₄⁺ from the slope of the linear curve: 

\[
\frac{1}{\text{influx}} = \frac{1}{I_{\text{max}} K_i + 1} + \frac{C}{I_{\text{max}}}
\]

The specific influx capacity was calculated as the maximal influx rate per unit of root dry mass. Substrate affinity (Kᵅ) of the HATS was calculated from the intercept divided by the slope of the linear curve. Kᵅ translates the efficiency to take up N inorganic compounds at low concentrations: a low Kᵅ value characterizes a high affinity of HATS for NO₃ or NH₄⁺.

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 NH₄⁺ and NO₃. 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 mm² g⁻¹, LDMC from 204 to 436 mg g⁻¹ and Nshoot from 2.5 to 4.3 mg g⁻¹ (Table 1). Variations for LDMC, SLA and Nshoot 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 Nshoot for species such as N. stricta, S. caerulea and F. paniculata, to an exploitative syndrome with high values of SLA and Nshoot 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 (Nshoot, SLA, LDMC and RDMC). Among traits, the strongest correlation was the negative one between
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, $r = -0.54$, $P < 0.001$; 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 $NO_3^-$ and $NH_4^+$ uptake**

The $I_{\text{max}}$ 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 $I_{\text{max}}$ expressed on a mass basis. The $I_{\text{max}}$ for both $NH_4^+$ and $NO_3^-$ differed between species (F-ratio = 6.02 and 3.90, $P$-value < 0.001 and < 0.01, respectively).
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 NH_4^+ and NO_3^– respectively, Fig. 3A, B). The I_max values for all species were higher for NH_4^+ (26–110 μmol h^{-1} g^{-1}) than for NO_3^– (3–27 μmol h^{-1} g^{-1}).

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 NH_4^+ or for NO_3^– (NH_4^+, r = -0.12 n.s.; 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
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 (Mommen 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 conservatism 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 Roninson, 1988; Hodge, 2004), hydroporic 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
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 (Poo 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 (Loulahia et al., 1999; Jumpponen et al., 2002; Warren, 2009). Our results highlighted higher NO\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+} between exploitative and conservative species, respectively (Maire et al., 2009), our results do not support such findings. The uptake rates for NO\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} and NO\textsubscript{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 (Lammers and Poorter, 1992; Nåsholm 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

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**Table 2. Pearson correlation between the species mean (n = 10) for functional traits measured for leaves (SLA, LDMC, N\textsubscript{shoot}), roots (SRL, RDMC, N\textsubscript{root}) and N uptake parameters (I\textsubscript{max} and K\textsubscript{m} for NH\textsubscript{4} and NO\textsubscript{3})**

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<th>Trait</th>
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<td>0.46</td>
<td>-0.32</td>
<td>0.55</td>
<td>0.58</td>
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<tr>
<td>NO\textsubscript{3} I\textsubscript{max}</td>
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<td>0.52</td>
<td>-0.34</td>
<td>-0.38</td>
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<tr>
<td>NH\textsubscript{4} K\textsubscript{m}</td>
<td>-0.38</td>
<td>0.68</td>
<td>-0.39</td>
<td>-0.47</td>
<td>-0.47</td>
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<td>0.34</td>
<td>-0.12</td>
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<tr>
<td>NO\textsubscript{3} K\textsubscript{m}</td>
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<td>0.82</td>
<td>-0.50</td>
<td>-0.18</td>
<td>0.17</td>
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<td>0.19</td>
<td>-0.17</td>
<td>-0.16</td>
<td>0.70</td>
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</table>

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

ACKNOWLEDGEMENTS

We wish to thank Anne-Françoise Ameline and Anne-Sophie Desfeux for assistance during plant culture, and Marie-Paule Bataille and Raphael Segura for IRMS analysis. The authors thank the ‘Conseil Régional de Basse-Normandie’ for the funding of a post-doctoral position for F.G. This study was conducted as part of the ERA-Net BiodivERsA project VITAL, ANR-08-BDVA-008.

LITERATURE CITED


