

1 Studies of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake ability of subalpine plants  
2 and resource-use strategy identified by their functional traits.

3 Legay N.<sup>1,2\*+</sup>, Grassein F.<sup>3,4\*</sup>, Arnoldi C.<sup>1</sup>, Segura R.<sup>3,4</sup>, Lâiné P.<sup>3,4</sup>, Lavorel S.<sup>1</sup>, Clément  
4 J.C.<sup>1,5</sup>.

5 \* Shared first co-authorship

6 + corresponding author: nicolas.legay@insa-cvl.fr

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8 <sup>1</sup> Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université Joseph Fourier, BP 53, 38041  
9 Grenoble Cedex 09, France

10 <sup>2</sup> INSA Centre Val de Loire, Université de Tours, CNRS, UMR 7324 CITERES, 37200 Tours,  
11 France

12 <sup>3</sup> Université de Caen Basse-Normandie, UMR 950 Ecophysiologie Végétale, Agronomie et  
13 nutritions N, C, S, Esplanade de la Paix, CS 14032, 14032 CAEN Cedex 5, France

14 <sup>4</sup> INRA, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de  
15 la Paix, CS 14032, 14032 CAEN Cedex 5, France

16 <sup>5</sup> CARTELE, UMR 0042 INRA-Univ. Savoie Mont Blanc, FR-73376, Le Bourget du Lac,  
17 France

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21 Running title: plant N uptake and functional strategy

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25 SUMMARY:

26 The leaf economics spectrum (LES) is based on a suite of leaf traits related to plant  
27 functioning and ranges from resource-conservative to resource-acquisitive strategies.  
28 However, the relationships with root traits, and the associated belowground plant  
29 functioning such as N uptake, including nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), is still  
30 poorly known. Additionally, environmental variations occurring both in time and in space  
31 could uncouple LES from root traits. We explored, in subalpine grasslands, the  
32 relationships between leaf and root morphological traits for 3 dominant perennial grass  
33 species, and to what extent they contribute to the whole-plant economics spectrum. We  
34 also investigated the link between this spectrum and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake rates, as well  
35 as the variations of uptake across four grasslands differing by the land-use history at peak  
36 biomass and in autumn. Although poorly correlated with leaf traits, root traits contributed  
37 to an economic spectrum at the whole plant level. Higher  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake abilities  
38 were associated with the resource-acquisitive strategy. Nonetheless,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake  
39 within species varied between land-uses and with sampling time, suggesting that LES and  
40 plant traits are good, but still incomplete, descriptors of plant functioning. **Although the**  
41  **$\text{NH}_4^+:\text{NO}_3^-$  uptake ratio was different between plant species in our study, they all showed**  
42 **a preference for  $\text{NH}_4^+$ , and particularly the most conservative species.** Soil environmental  
43 variations between grasslands and sampling times may also drive to some extent the  $\text{NH}_4^+$   
44 and  $\text{NO}_3^-$  uptake ability of species. Our results support the current efforts to build a more  
45 general framework including above- and below-ground processes when studying plant  
46 community functioning.

47 Keywords: Leaf and root traits, Nitrate and ammonium uptake, plant assimilation, Resource  
48 use strategy, Subalpine grasslands

## 49 Introduction

50 Functional traits have been widely used to describe different plant strategies. One major axis  
51 of specialisation involves a trade-off between conservation of resources in well protected and  
52 long lived tissues, and acquisition of resources by tissue with high use-efficiency and turn-  
53 over, and commonly referred as the leaf economic spectrum (LES, Wright *et al.* 2004). More  
54 specifically, species with an exploitative strategy share similar leaf attributes such as high  
55 specific leaf area (SLA) and nitrogen concentrations (LNC) that have been associated with  
56 short leaf life-span, high photosynthetic capacity as well as high decomposability (Reich  
57 2014, Cornwell *et al.* 2008), and dominate in nutrient rich environments, while slow-growing  
58 conservative species carry opposite trait values and are more common in poor or harsh  
59 conditions (Chapin 1980, Ordóñez *et al.* 2009). Despite some evidences of a similar  
60 contribution of root traits to the plant strategy (Roumet *et al.* 2006, Freschet *et al.* 2010, Fort  
61 *et al.* 2013), the significance of root traits is less understood than the one for leaf traits, mainly  
62 because weak correlations between analogous leaf and root traits have been reported (Craine  
63 *et al.* 2005, Tjoelker *et al.* 2005, Freschet *et al.* 2010), and also because root functioning is  
64 often overlooked compared to leaves in field conditions.

65 Nutrient uptake ability, one of the main functions provided by roots (Hodge 2004, James *et al.*  
66 2009), is both influenced by anatomical and physiological adjustments such as specific root  
67 length or maximal uptake rate ( $V_{max}$ , but see Bassirirad 2000). Among nutrients, nitrogen is  
68 one of the best studied mineral nutrients and its uptake by plants under both the ammonium  
69 ( $NH_4^+$ ) and nitrate ( $NO_3^-$ ) forms is influential for plant and ecosystem functioning. However,  
70 rarely have morphological and physiological properties of root been assessed simultaneously  
71 in field conditions, whereas some information come from species grown in standardized  
72 conditions (Maire *et al.* 2009, Grassein *et al.* 2015).  $NH_4^+$  and  $NO_3^-$  uptake can indeed be  
73 influenced by several environmental factors justifying the use of controlled conditions to

74 estimate uptake parameters in a comparative purpose. For example,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake has  
75 been reported to vary in response to temperature or pH (Garnett and Smethurst 1999).  
76 Nevertheless,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake ability also differs between species, and is partially  
77 related to plant strategy and their functional traits (Grassein *et al.* 2015), but these results need  
78 to be validated for plant grown in natural conditions. Finally,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  transporters have  
79 two components: a constitutive component and a component induced by the presence of  $\text{NH}_4^+$   
80 and  $\text{NO}_3^-$  in the soil solution. Thus, it is important to study interspecific differences for  $\text{NH}_4^+$   
81 and  $\text{NO}_3^-$  uptake at a given site. Otherwise, it is difficult to interpret differences as resulting  
82 from species differences.

83 Subalpine grasslands are subject to the combined effects of climate and anthropogenic factors,  
84 both influencing N cycling and thus N availability for organisms (Bardgett *et al.* 2005, Legay  
85 *et al.* 2013). Decreased management intensity favours plant species with resource  
86 conservative traits (Quétier *et al.* 2007), which are usually associated with fungal-dominated  
87 belowground communities (de Vries *et al.* 2012, Grigulis *et al.* 2013). Concomitantly, it slows  
88 down N cycling (Zeller *et al.* 2000, Robson *et al.* 2010), favouring the accumulation of soil  
89 ammonium ( $\text{NH}_4^+$ ) rather than soil nitrate ( $\text{NO}_3^-$ ) (Robson *et al.* 2007). Plants growing in such  
90 variable conditions are likely to adjust their N uptake ability, as it has been shown for  
91 functional traits (Quétier *et al.* 2007, Grassein *et al.* 2015).

92 In this study, we investigated the relationships between functional traits and inorganic N  
93 ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) uptake for three perennial grass species with contrasted leaf economic  
94 strategies. Because soil inorganic  $\text{NH}_4^+$  and  $\text{NO}_3^-$  availability and plant  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake  
95 ability are likely to vary across seasons and in response to management (Jaeger *et al.* 1999,  
96 Miller *et al.* 2009), we examined these relationships for individuals occurring in four  
97 subalpine grasslands with different management and throughout the growing season, thereby  
98 testing their temporal consistency. Estimating root  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake, and measuring  
99 functional traits for leaves and roots, we tested the following hypotheses: (1) **similar to leaf**

100 traits, root traits are also contributing to the plant economics spectrum with root traits  
101 reflecting nutrient acquisition (e.g. high specific root length and root nitrogen content)  
102 expected to be more associated to the exploitative syndrome, (2) and with more exploitative  
103 species being more efficient to take up both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . (3) As functional traits are  
104 influenced by environmental conditions, we hypothesised that  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake will be  
105 influenced by environmental variations between grasslands, as well as during the growing  
106 season, probably following  $\text{NH}_4^+$  and  $\text{NO}_3^-$  availability depending on the most abundant form.

107

108 Material and methods

109 Study site and species

110 The study site is located in the upper Romanche valley of the central French Alps between the  
111 village of Villar d'Arène and the Lautaret Pass (Table 1). The climate is subalpine with a  
112 strong continental influence. Winters are cold and snowy, with monthly average minimum  
113 temperatures of  $-15.9^\circ\text{C}$  in February, maximum monthly average temperature of  $23.8^\circ\text{C}$  in  
114 July, and mean annual precipitation of 956mm (unpublished data, [sajf.ujf-grenoble.fr](http://sajf.ujf-grenoble.fr)). The  
115 growing season starts following snow melt in late April - early May and continues until late  
116 September or October depending on the date of the first snow in autumn.

117 Given the hypothesis that  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake could be an important hard plant trait related  
118 to resource use strategy (as suggested by soft structural and morphological traits) and to field  
119 dominance, and due to the degree of precision chosen for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake estimations  
120 (see 2.2), a compromise was necessary regarding the number of species, grasslands and  
121 replicates to be investigated. This sampling adjustment was required to conduct N uptake  
122 estimations for all individuals in a brief enough time period so that most abiotic factors  
123 remained as comparable as possible (soil moisture, temperature, radiation).

124 We chose three common and dominant grass species, *Dactylis glomerata* L., *Bromopsis*  
125 *erecta* (Huds.) Fourr. (formerly *Bromus erectus* (Huds.)) and *Patzkea paniculata* (L.)  
126 G.H.Loos (formerly *Festuca paniculata* (L.) Schinz & Thell.). All species are perennial,  
127 arbuscular mycorrhizal non-dependent species and span a gradient from more exploitative (*D.*  
128 *glomerata*) to more conservative (*F. paniculata*) resource use strategies (Grassein et al. 2015).  
129 Four grasslands (Table 1), described in Quétier *et al.* (2007), were chosen for their contrasting  
130 past and current managements, and were similar to the grasslands studied by Robson *et al.*  
131 (2007, 2010) : (i) Terraced Mown and Fertilized (TMF), (ii) Terraced Unmown not fertilized  
132 but lightly grazed (TU), (iii) Un-terraced Mown grassland (UM) and (iv) Un-terraced  
133 Unmown but lightly grazed grassland (UU), representing a gradient of decreasing  
134 management intensity. To reflect field dominance patterns, *D. glomerata* was sampled in  
135 TMF, *B. erectus* in TU, *F. paniculata* in UM, and all three species were sampled in UU where  
136 they coexist, although *F. paniculata* was dominant (Table 1).

137 To assess  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake patterns over the growing season, the same sampling design  
138 was repeated twice during 2010. At each date for each species and grassland, we sampled the  
139 roots and soil (approximately: 25x25x25 cm) of five individuals (genetically distinct  
140 individuals at least 2m apart). The first sampling corresponded to the peak biomass and  
141 targeted flowering onset (just before anthesis), and the second sampling corresponded to  
142 autumn after last management activities occurred. For *D. glomerata* in TMF and *B. erectus* in  
143 TU, the two sampling dates were mid-June and mid-September. For *F. paniculata* in UM and  
144 the three species in UU, the sampling dates were: early July and early September. These two  
145 dates are called "Summer" and "Autumn" hereafter. As much as possible, species were  
146 sampled at the same time **during** the day to avoid any diurnal variation in N uptake (Gessler et  
147 al. 1998). In total, we have sampled 12 points (3 species\*2 seasons\*2 habitats per species).

148

149 Soil nitrogen pools

150 At each date and for each grassland, soil nitrogen concentrations were measured from six soil  
151 cores (dimensions 4.5 cm Ø, 10 cm deep) kept on ice in the field and maintained at 4°C upon  
152 return to the laboratory (within 2h). Soils were sieved through a 5.6 mm mesh to remove roots  
153 and stones. A subsample of 10g fresh sieved soil was prepared for extraction of inorganic N in  
154 0.5M K<sub>2</sub>SO<sub>4</sub>, and analysed using a colorimetric analyser (FS-IV autoanalyser (OI-Analytical,  
155 College Station, TX, USA) (following Bowman *et al.* 2003) to measure soil concentrations of  
156 ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and Total Dissolved Nitrogen (TDN). Soil aliquots were  
157 used to determine soil water (7 days at 70°C) and soil organic matter contents (550°C during  
158 4 hours). Finally, soil subsamples were air-dried to measure soil pH, or ground to a fine  
159 powder for measurements of total carbon (C) and N contents using an elemental analyser  
160 (FlashEA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA).

161 At each date, five individuals of each species, with roots and soil, were excavated from each  
162 field, transferred within half an hour to the laboratory located at the Lautaret Pass (Station  
163 Alpine Joseph Fourier) and kept at 4°C until the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> uptake rate measurements to  
164 maintain the functional integrity of the roots. Living young fine roots were washed with  
165 deionised water, cut to 2-cm length and then, rinsed in 1mM CaSO<sub>4</sub> at 4°C for 3 min. The  
166 NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> uptake rates were measured during the first hour following plant harvest as  
167 described by Louahlia *et al.* (2000). The optimal conditions for uptake measurements by  
168 excised root determined by Lainé *et al.* (1993) were used in the present study.

169

170 Functional traits

171 Functional traits were measured for roots and leaves using standardised protocols (Perez-  
172 Harguindeguy *et al.* 2013). Two of the individual root sub-samples were used to estimate root

173 dry matter content (RDMC), specific root length (SRL, Winrhizo® software, fresh length per  
174 unit of dry mass), and were further analysed to obtain root  $^{15}\text{N}$  natural abundance and root  
175 nitrogen concentration (RNC, N mass per unit of dry mass). Specific leaf area (SLA, fresh  
176 area per unit of dry mass), leaf and root dry matter contents (LDMC and RDMC, dry mass per  
177 unit of fresh mass), leaf nitrogen concentration (LNC, N mass per unit of dry mass) were also  
178 measured.

179 Nitrogen uptake estimation: the “excised” roots method

180 Although measuring only a net N uptake, which is the result of influx and efflux, the direct  
181 measurement of N uptake using excised roots allows characterising the plant uptake kinetics  
182 for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  while controlling for the environmental variations. This makes it possible  
183 to compare different species at the cost of losing relevant ecological information (Lucash *et*  
184 *al.* 2007). This method was thus applied to plants collected in the field. Root N uptake  
185 kinetics started within 60 min after excision, thereby avoiding the potential decline in N  
186 uptake ability reported to start after 3h (Louahlia *et al.* 2000). Nitrate and ammonium uptake  
187 by plants involved mainly the transport system called HATS (High Affinity Transport  
188 System). It contributes to N uptake at low to moderate concentrations of external N (<1mM)  
189 and saturates at 0.2-0.5 mM (Kronzucker *et al.* 1999, Min *et al.* 2000), which makes it the  
190 more likely system used by plants growing in natural and semi-natural ecosystems limited by  
191 N (Bassirirad 2000, Maire *et al.* 2009). The estimation of the maximum  $\text{NH}_4^+$  and  $\text{NO}_3^-$   
192 uptake rates by HATS requires a range of N concentrations below 1mM at which the  $V_{\text{max}}$   
193 can be reached depending on species (Grassein *et al.* 2015). Consequently, uptake was  
194 estimated from the accumulation of  $^{15}\text{N}$  in root sub-samples incubated for one hour in a buffer  
195 solution (pH = 5.5- following Leon *et al.* (1995)), containing a range of N concentrations (20,  
196 50, 100, 250, 500 and 1000  $\mu\text{M}$ ). Six sub-samples were incubated in  $\text{K}^{15}\text{NO}_3$  and the other six  
197 in  $(^{15}\text{NH}_4)_2\text{SO}_4$  with a  $^{15}\text{N}$  excess of 99% atom. The two N forms were tested individually in



198 order to avoid possible interactions (Kronzucker *et al.* 1999). Solution volumes and fresh  
199 weights were selected to avoid N depletion during the experiment. After 1h incubation, roots  
200 were washed twice for one minute with a 1mM CaSO<sub>4</sub> at 4°C to stop any metabolic  
201 processes. Roots were then dried at 60°C for 72h, ground to a fine powder and analysed by  
202 IRMS at the University of Caen (Isoprime GV instruments, Stockport, UK) to obtain <sup>15</sup>N  
203 Atom% and N concentrations.

204

205 Data analysis

206 Nitrogen Uptake Rate (NUR) was calculated for each concentration and each inorganic N  
207 form (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) using the <sup>15</sup>N increase in the root incubated compared to the non-  
208 incubated control, and expressed by unit of time and dry mass (nmolN.h<sup>-1</sup>.g<sup>-1</sup> of dry roots, see  
209 Leon *et al.* 1995). The dependence of NUR on substrate concentration was fitted for each  
210 individual and Hanes's relation (Michaelis transformation) was used to estimate the maximum  
211 uptake rate (Vmax) defined as the maximum NUR for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (Leon *et al.* 1995).  
212 Finally, the NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> uptake ratio was calculated as the ratio between NH<sub>4</sub><sup>+</sup> Vmax and NO<sub>3</sub><sup>-</sup>  
213 Vmax.

214 A principal component analysis (PCA) was performed using all plant functional traits at the  
215 individual level to describe their functional strategy based on leaf and root traits. To  
216 investigate the relationships between functional traits of leaves and roots, and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>  
217 uptake ability (hypothesis 1), we used Pearson correlation coefficients. Relationships between  
218 the functional strategy and uptake of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> at the root level were tested using  
219 regression analyses between the N uptake rates (Vmax) and the first PCA.

220 Comparisons of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> uptake rates for species (hypothesis 2), fields and date  
221 (hypothesis 3) were conducted with ANOVA followed by Tukey tests to compare species and

222 grasslands. In details, the effects of sampling time and fields on plant traits within each  
223 species were tested using two-ways ANOVA. Similarly, the effects of sampling time and  
224 fields on maximal  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake rates within each species were tested using two-  
225 ways ANOVA. The effects of fields and sampling time on  $\text{NH}_4^+:\text{NO}_3^-$  ratio within each  
226 species were tested using two-ways ANOVA. Then, we tested only in UU grasslands, the  
227 species effect using one-way ANOVA. Finally, we used a two-ways ANOVA and Tukey post  
228 hoc test to test soil parameters differences between fields and dates. Data were log-  
229 transformed when necessary to achieve normality and heteroscedasticity. All statistical  
230 analyses were performed using the software R 3.4.4, with multivariate analyses (PCA) being  
231 performed using the package Ade4 (Dray & Dufour 2007).

232

## 233 Results

234 We observed large variations for leaf and root functional traits in spite of a restricted number  
235 of species in our study (Table 2). The range of variation was similar to, and sometimes even  
236 higher than the variability reported in Fort *et al.* (2013) for a larger set of species occurring in  
237 a similar ecosystem, including *D. glomerata* and *B. erectus*. The PCA of functional traits  
238 highlighted a first axis explaining 62.1% of the total variance (Fig. 1). The three species  
239 differed significantly for their mean position along this axis ( $p=0.012$ ), with positive values  
240 for *D. glomerata* and negative values for *F. paniculata*. Positive values along this axis were  
241 characterised by high SLA, LNC and SRL, and low LDMC. Among these, SLA and LNC  
242 have been reported as major contributors to a resource economic spectrum establishing the  
243 existence of a fundamental trade-off between plant features allowing resource capture and  
244 those allowing resource conservation.

245 This functional axis was positively correlated to  $\text{NH}_4^+$  and  $\text{NO}_3^-$   $V_{\text{max}}$  (Fig 2a and 2b) and  
246 negatively to  $\text{NH}_4^+:\text{NO}_3^-$  uptake ratio (Fig 2c) indicating a more pronounced preference for  
247  $\text{NH}_4^+$  at lower values of axis 1. Except RDMC, all traits taken separately were poorer  
248 predictors of the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  maximum uptake rates than this functional axis, although the  
249 first PCA axis was significantly correlated with all functional traits (Table 3).

250 In UU grassland,  $\text{NH}_4^+V_{\text{max}}$  in summer was similar for the three species (Fig. 3a) but greater  
251 for *D. glomerata* for  $\text{NO}_3^- V_{\text{max}}$  ( $p < 0.001$ , Fig. 3b).  $V_{\text{max}}$  in autumn for both N forms was  
252 lower for *F. paniculata* compared to the two other species ( $\text{NH}_4^+ p < 0.05$ ,  $\text{NO}_3^- p < 0.001$ ).  
253 Comparing the different grasslands within species, we observed reduced  $\text{NO}_3^-$  and  $\text{NH}_4^+$   
254  $V_{\text{max}}$  values in the UU grassland for *D. glomerata* (in summer and in autumn) and *B. erectus*  
255 (summer) compared to the other grasslands. On the other hand, highest  $\text{NH}_4^+ V_{\text{max}}$  for *B.*  
256 *erectus* and *F. paniculata* were found in UU during the autumn. Illustrating the seasonal  
257 variability, all species in the UU grassland had higher  $\text{NH}_4^+$  maximum uptake rates in the  
258 autumn than in the summer, as well as higher  $\text{NO}_3^-$  uptake for *B. erectus*.  $\text{NH}_4^+:\text{NO}_3^-$  uptake  
259 ratio did not vary in time, but always showed higher values in the UU for the three species  
260 compared to the other grasslands, and overall greater values for *F. paniculata* (Fig. 4).

261 Within species, a limited number of traits were significantly different between grasslands  
262 (Table 2). We only observed significant differences in autumn, with highest LDMC in TU for  
263 *B. erectus*, highest LDMC and RDMC in UU for *D. glomerata*, and highest LDMC but lowest  
264 SRL for *F. paniculata* in UM. However, changes in response to the season were more  
265 consistent among species and grasslands, with an increase of LDMC and a decrease of SLA in  
266 autumn compared to the summer. We also observed higher LNC for *D. glomerata* and *F.*  
267 *paniculata* during the summer than during the autumn in TMF and UM respectively, and  
268 higher RDMC during the autumn for *D. glomerata* in UU.

269 Since all species occurred in the UU grasslands, we choose to focus on soil parameters from  
270 **these** grasslands. UU and UM only differ for SWC in autumn (Table 4), all other soil  
271 variables were similar between these two grasslands, which had similar past land-use history  
272 (Table1). UU had consistently higher SWC and SOM, and lower soil pH and CN ratio than  
273 TMF and TU. All grasslands had similar soil  $\text{NH}_4^+$  concentrations. During the summer, we  
274 observed higher TDN and  $\text{NH}_4^+:\text{NO}_3^-$  soil ratio, and lower soil  $\text{NO}_3^-$  concentration in UU  
275 compared to TMF and TU, but we did not find these differences in autumn.

276

277 Discussion:

278 Relationships between leaf and root traits

279 In the aim to find parallels between above and below-ground organs (e.g. Roumet et al. 2006),  
280 several studies have investigated the relationships between analogous traits measured for  
281 leaves and roots. While positive relationships have been reported for SLA vs SRL (Craine &  
282 Lee 2003, Craine et al. 2005, Freschet et al. 2010), other studies have reported a lack of  
283 relationships between SLA vs SRL (Craine et al. 2001, Tjoelker et al. 2005). In our study, we  
284 did not find any relationships between SRL/SLA, LDMC/RDMC and LNC/RNC, and this  
285 could be related to our limited number of species/replicates. Nevertheless, we observed trade-  
286 offs at the leaf and root levels between traits, namely N concentration and dry matter content.  
287 **Such traits correlations between** the leaf and root levels **have** already been reported (Freschet  
288 et al. 2010), **though** relatively **weak** relationships **were found here** between analogous traits  
289 belowground and aboveground. Different selective pressures for leaf and root traits as well as  
290 specialisations for the acquisition of different resources (e.g. light vs nutrient) could explain  
291 this absence of association between belowground and aboveground traits (Craine et al. 2005,  
292 Liu et al. 2010), while the global strategy at the plant level could remain the same since high

293 efficiency for light or for nutrients could be related to the same physiological adaptation, as  
294 pointed out previously for stress tolerance (Chapin 1980). Although we found that leaf  
295 functional traits (LNC) can be correlated with root  $\text{NH}_4^+$  and  $\text{NO}_3^-$  maximal uptake rate as  
296 previously shown (Osoné et al. 2008; Maire et al. 2009), here root traits (RDMC, SRL)  
297 appeared to be more related to  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake rates (Rewald et al. 2014), even if  
298 deeper understanding of the relationship between root traits and nutrient acquisition remains  
299 needed (Roumet et al. 2016). **The interpretation is however limited here by the fact that only**  
300 **three subalpine herbaceous species were studied.**

301

302 Relationship between N maximum uptake rate ( $V_{\text{max}}$ ) and plant strategy

303 Our results showed that a stronger exploitative syndrome (higher SRL, SLA, LNC and lower  
304 RDMC) was associated with higher  $V_{\text{max}}$  for both inorganic N forms, rejecting the  
305 hypothesis of a trade-off between maximum uptake rate of each N forms. **Ammonium toxicity**  
306 **has been reported for some plant species (review in Britto & Kronzucker 2002), as well as**  
307 **negative interactions between the uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Kronzucker et al. 1999), and this**  
308 **could promote a trade-off in the acquisition of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  between species (Maire et al.**  
309 **2009). Here, we estimated  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake independently to avoid such interactions**  
310 **during measurements, and our results did not support a trade-off but rather suggest a**  
311 **synergistic uptake of both N forms. Provision of  $\text{NO}_3^-$  has been demonstrated to alleviate the**  
312  **$\text{NH}_4^+$  toxicity (Britto & Kronzucker 2002), and even to favour  $\text{NH}_4^+$  uptake. We indeed**  
313 **observed higher uptakes for  $\text{NH}_4^+$  than for  $\text{NO}_3^-$ , indicating a preference of all species for**  
314  **$\text{NH}_4^+$ , especially for individuals with a more conservative syndrome of traits. **This is likely to****  
315 **be related to the lower energetic cost for plant species to uptake and assimilate  $\text{NH}_4^+$**   
316 **compared to  $\text{NO}_3^-$  (Salsac et al. 1987). Besides, more exploitative plants have a lower**  
317 **preference for  $\text{NH}_4^+$  compared to more conservative individuals, but expressed higher**

318 maximal uptake rates than more conservative individuals for both N forms. **At the grassland**  
319 **plant community scale, this  $\text{NH}_4^+$  vs.  $\text{NO}_3^-$  preference is likely to have consequences on**  
320 **ecosystem functioning and N balance ; for instance because  $\text{NO}_3^-$  is more prone to leaching**  
321 **whereas  $\text{NH}_4^+$  is better retained in soil (Boudsocq et al. 2012).** Overall, our results suggest that  
322 changes in functional leaf traits related to a higher potential photosynthesis efficiency and  
323 light capture appeared to be associated at the root level with higher maximal uptake rates for  
324 both N forms.

325 Nitrogen uptake variations in response to management and sampling dates

326 Nitrogen uptake rate is usually considered as a property of plant species, but little is  
327 known about variation in within-species N uptake rates in grasslands with different land-use  
328 history and at different times during the growing season. In our study, we observed that  
329 nitrogen uptake rates could differ strongly for the same species in different grasslands (e.g. *B.*  
330 *erectus* and *D. glomerata* in the UU grassland). On the other hand, the time of the year also  
331 influenced the N uptake rates of all species, with for example **a higher  $\text{NH}_4^+$  uptake in the**  
332 **autumn than in summer in UU grasslands, whereas no difference was detected in UU**  
333 **grasslands for *B. erectus*.** Overall, grasslands were weakly discriminated by functional traits,  
334 suggesting that other factors such as soil parameters may explain the within species N uptake  
335 differences between grasslands.

336 Nitrogen uptake can vary depending on the amount of N available in the soil (Gavito  
337 et al. 2001). Soil  $\text{NH}_4^+$  concentration, the main N source taken up by plants in our study, was  
338 similar in the four investigated grasslands, whereas a higher soil total dissolved N (TDN) was  
339 measured in the UU grassland. Consequently, the lower N uptake rates observed in this UU  
340 grassland cannot be explained by a lower N availability. As reported by previous studies,  
341 subalpine grasslands can show the legacy effects of former management activities, leading to  
342 slower N cycling (Zeller et al. 2000, Robson et al. 2007). Indeed, we observed lower pH and

343 higher soil water and organic matter contents in the UU grassland suggesting variations in N  
344 cycling and in the quality of the available N, not only in its quantity (Garnett & Smethurst  
345 1999, Robson et al. 2010). Supporting this hypothesis, we observed variations in soil  $\text{NO}_3^-$   
346 concentrations, and consequently soil  $\text{NH}_4^+:\text{NO}_3^-$  ratio, between the studied grasslands.  
347 Although we could not directly relate *in situ* soil parameters to N plant uptake estimated under  
348 “controlled” conditions, we interestingly observed parallel changes for  $\text{NH}_4^+$  uptake rates and  
349 soil  $\text{NH}_4^+:\text{NO}_3^-$  ratio in grasslands where individuals have been sampled. For example, both  
350 *B. erectus*  $\text{NH}_4^+$  uptake and soil  $\text{NO}_3^-$  concentration were lower during the summer and higher  
351 during the autumn in TU than in UU.

352 Rarely investigated in natural ecosystems, experimental evidences on cultivated plants  
353 have demonstrated the effects of soil  $\text{NH}_4^+:\text{NO}_3^-$  concentration ratio on plant N uptake  
354 (Errebhi & Wilcox 1990, Bar-Tal et al. 2001). Yet, the effects were largely species-dependent  
355 and trade off were sometime reported between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptakes (Warncke & Barber  
356 1973, Kronzucker et al. 1999, Maire et al. 2009). The preferential uptake for an inorganic N  
357 form could also be influenced by environmental and physiological factors (Britto &  
358 Kronzucker 2013). Our results did not support any trade-off in the intrinsic ability of plant  
359 species to take up both N forms, even after removing possible environmental conditions or  
360 interactions between inorganic N forms. Although we could not directly test for the  
361 relationship between soil parameters and plant N uptake rates, differences between grasslands  
362 in the N uptake within species highlight that management practices may have important  
363 effects on plant N uptake, likely through N cycling changes and the quality of the N pool  
364 available as already pointed out by previous studies (Zeller et al. 2000, Robson et al. 2007).  
365 Other studies have suggested that N preference could be dependent on the soil availability of  
366 the different N forms (Näsholm et al. 2009, Stoelken et al. 2010). While our results partially  
367 supported this hypothesis, with variation within species between different grassland, the

368 different species sampled in the same grassland showed differences in their  $\text{NH}_4^+:\text{NO}_3^-$  uptake  
369 ratio, supporting the hypothesis that this “preference” is partially related to the strategy of  
370 species, or at least to species identity. But overall, more exploitative species with higher  
371 maximum uptake rates for one inorganic N form are also likely to have high uptake rates for  
372 other N forms as previously found (Kastovska & Santruckova 2011).

373 Nevertheless, the plant preference for N forms is a complex topic (Britto &  
374 Kronzucker 2013), and careful considerations should be given to the environmental  
375 conditions where the species occur. Since N cycling is controlled by a large set of parameters  
376 including pH, soil moisture, land-use, short and long-term variations in the predominant N  
377 forms available for plants are to be expected. Under harsh conditions, plants can also take up  
378 organic N (amino acids) directly and/or through fungi (Näsholm et al. 2000). While we  
379 assumed that this source of N is of limited importance for our species in our relatively fertile  
380 grasslands (Kahmen et al. 2009), a full understanding of the N preference, and discussion  
381 about species coexistence through N forms sharing, would require careful investigations,  
382 beyond the possibility in our study. Nonetheless, the variability we observed in the ratio of  
383 uptake between the inorganic N forms suggested that, to some extent, plant physiology was  
384 adjusted to match the soil conditions where species occurred. Yet, differences between  
385 species with different strategies remain, with higher uptake rate for both N forms associated  
386 with a more exploitative strategy, and we hypothesised that this should be also the case for  
387 organic N sources (Kastovska and Santruckova 2011). Nevertheless, we acknowledge that this  
388 question could be more important in harsh environments where soil organic N is relatively  
389 more abundant as a N source for plants (Mozdzer et al 2014). Further investigations remain  
390 needed on the variations of plant N uptake under field conditions, in link with potential  
391 variations in N cycling in response to land-use or during the season (Robson et al. 2010,  
392 Legay et al. 2013).



393

394 Variations of N uptake ability during the growing season

395 Plant N uptake ability also varies during the growing season, with N uptake increasing  
396 (Stahl et al. 2011) or decreasing (Jaeger et al. 1999) depending on the ecosystems  
397 investigated. In the UU grassland,  $\text{NH}_4^+$  uptake was higher for all species during the autumn  
398 than during the summer, and the same was found for  $\text{NO}_3^-$  uptake by *B. erectus*. Plant activity  
399 is usually considered to slow down during the autumn compared to the peak biomass in  
400 summer, an assumption supported by higher LDMC and lower SLA for all species related to  
401 the senescence of leaves. However, we did not observe any changes for root traits, suggesting  
402 that roots could remain active during this time of the growing season, especially in the process  
403 of resource storage, an important feature for subalpine/alpine plants (Kleijn et al. 2005).  
404 Additionally, studies have reported an increase of grassland N cycling rate in the autumn that  
405 could be explained by more favourable soil conditions (first rains and mild temperature), and  
406 associated with still active N uptake by plants as observed in our study (Miller et al. 2009,  
407 Larsen et al. 2012). **This could also be related to the better retention of  $\text{NH}_4^+$  vs.  $\text{NO}_3^-$  in wet  
408 soils during autumn, making  $\text{NH}_4^+$  more available for plant uptake (Brady and Weil 2001).**  
409 Despite the fact that only few soil parameters differed between the two investigated seasons in  
410 the UU grassland, the N uptake increase in autumn was more likely a site-dependent effect  
411 related to soil conditions (Miller et al. 2009, Stahl et al. 2011, Legay et al. 2013), rather than a  
412 species response since all species did show the same pattern in the other grasslands. Yet, a  
413 multiple-year study remains necessary to better conclude on these seasonal patterns.

414

415 Conclusions

416 By estimating inorganic root N uptake under controlled conditions from plants grown up  
417 under field conditions, our results support the assumption that root and leaf functional traits  
418 are associated with the ability of plants to acquire soil inorganic N. In particular, the observed  
419 pattern for roots characteristics appeared similar to the one observed in the leaf economic  
420 spectrum, with higher inorganic N uptake rates associated with more exploitative syndrome of  
421 traits. However, a weak relationship between leaf and root traits suggests that leaf traits alone  
422 were insufficient to predict inorganic N uptake. Additionally, inorganic N uptake varied  
423 within species during the growing season and in response to local conditions, making root  
424 traits and soil parameters important features of the relationships between plant functioning  
425 and grasslands N cycling. **Nevertheless, these results based on excised root study need to be**  
426 **confirmed at the whole plant level using, for instance,  $^{15}\text{N}$  labelling.**

427

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620 **Table 1:** Description of the studied grasslands. Past and current land uses describe the former  
621 and current management of these grasslands (see Quetier et al. 2006 for more information).  
622 TMF: Terraced Mown and Fertilized, TU: Terraced Unmown not fertilized but lightly grazed,  
623 TM: Unterraced Mown, UU: Unterraced Unmown but lightly grazed.

624

Field

label	GPS coordinates	Sampled species	Past Land Use	Current Land Use	Elevation
TMF	45° 2'42.77"N 6°20'35.29"E	<i>Dactylis glomerata</i>	Arable rotation	fertilized hay meadow	1800m
TU	45° 2'24.74"N 6°21'38.15"E	<i>Bromus erectus</i>	Arable rotation	grazed pasture (sheep)	1840m
UM	45° 1'58.59"N 6°23'0.80"E	<i>Festuca paniculata</i>	Hay meadow	unfertilized hay meadow	1980m
UU	45° 1'55.76"N 6°23'1.57"E	<i>Festuca paniculata</i> <i>Bromus erectus</i> <i>Dactylis glomerata</i>	Hay meadow	grazed pasture (sheep)	1960m

625

626

**Table 2:** Mean values  $\pm$  standard errors of leaf and root traits for each species, site and sampling time (n=5). For a given trait and species, statistically similar values have the same letter (Tukey post-hoc test). Bold values indicate the season with the highest trait values for a given species in a given grassland.

Species	Site	Season	SLA (mm <sup>2</sup> .g <sup>-1</sup> )	LDMC (mg.g <sup>-1</sup> )	LNC (mg.g <sup>-1</sup> )	SRL (m.g <sup>-1</sup> )	RDMC (mg.g <sup>-1</sup> )	RNC (mg.g <sup>-1</sup> )
<i>B. erectus</i>	TU	Summer	<b>30.07 <math>\pm</math> 0.8<sup>a</sup></b>	277.6 $\pm$ 2.1 <sup>c</sup>	21.46 $\pm$ 0.2	249.86 $\pm$ 72.0	307.77 $\pm$ 22.4	6.06 $\pm$ 0.1
		Autumn	15.43 $\pm$ 1.1 <sup>c</sup>	<b>444.06 <math>\pm</math> 16.8<sup>a</sup></b>	12.09 $\pm$ 0.9	253.58 $\pm$ 34.3	303.5 $\pm$ 6.3	7.68 $\pm$ 0.4
	UU	Summer	<b>22.58 <math>\pm</math> 0.7<sup>b</sup></b>	305.06 $\pm$ 4.2 <sup>c</sup>	15.57 $\pm$ 0.8	328 $\pm$ 74.2	290.82 $\pm$ 21.7	7.17 $\pm$ 0.1
		Autumn	16.37 $\pm$ 1 <sup>c</sup>	<b>385.1 <math>\pm</math> 11.5<sup>b</sup></b>	19.28 $\pm$ 2.7	222.12 $\pm$ 75.4	288.32 $\pm$ 21.6	7.87 $\pm$ 0.5
<i>D. glomerata</i>	TMF	Summer	<b>34.06 <math>\pm</math> 1.6<sup>a</sup></b>	248.79 $\pm$ 17.7 <sup>c</sup>	<b>32.6 <math>\pm</math> 3.4<sup>a</sup></b>	426.22 $\pm$ 60.5	232.49 $\pm$ 20.5 <sup>b</sup>	7.28 $\pm$ 0.4
		Autumn	20.15 $\pm$ 2 <sup>b</sup>	<b>298.51 <math>\pm</math> 2.5<sup>b</sup></b>	19.41 $\pm$ 2.2 <sup>b</sup>	290 $\pm$ 48.6	249.09 $\pm$ 8 <sup>b</sup>	7.99 $\pm$ 0.3
	UU	Summer	26.17 $\pm$ 0.3 <sup>ab</sup>	265.78 $\pm$ 9.1 <sup>bc</sup>	<b>24.2 <math>\pm</math> 1.4<sup>ab</sup></b>	318.69 $\pm$ 63.8	254.08 $\pm$ 5.6 <sup>b</sup>	6.91 $\pm$ 0.5
		Autumn	24.34 $\pm$ 1.7 <sup>b</sup>	<b>389.69 <math>\pm</math> 10.5<sup>a</sup></b>	21.81 $\pm$ 2.5 <sup>ab</sup>	216.87 $\pm$ 44.7	<b>304.99 <math>\pm</math> 8.8<sup>a</sup></b>	7.16 $\pm$ 0.2
<i>F. paniculata</i>	UM	Summer	<b>23.26 <math>\pm</math> 0.4<sup>a</sup></b>	232.06 $\pm$ 0.6 <sup>c</sup>	<b>20.22 <math>\pm</math> 1.9<sup>a</sup></b>	195.85 $\pm$ 21.3 <sup>ab</sup>	300.1 $\pm$ 21.5	5.15 $\pm$ 0.4
		Autumn	8.78 $\pm$ 0.5 <sup>b</sup>	<b>433.87 <math>\pm</math> 18.8<sup>a</sup></b>	10.61 $\pm$ 1.4 <sup>b</sup>	153.37 $\pm$ 20.5 <sup>b</sup>	369.12 $\pm$ 21.7	4.32 $\pm$ 0.5
	UU	Summer	<b>21.36 <math>\pm</math> 0.9<sup>a</sup></b>	261.06 $\pm$ 15.6 <sup>c</sup>	14.52 $\pm$ 1.8 <sup>ab</sup>	166.21 $\pm$ 20.7 <sup>ab</sup>	380.91 $\pm$ 6.4	4.16 $\pm$ 0.1
		Autumn	11.76 $\pm$ 1.4 <sup>b</sup>	<b>369.71 <math>\pm</math> 7.2<sup>b</sup></b>	15.53 $\pm$ 3.6 <sup>ab</sup>	293.71 $\pm$ 54.5 <sup>a</sup>	366.84 $\pm$ 23.3	4.31 $\pm$ 0.1



**Table 3.** Pearson correlations between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  maximum uptake rates ( $V_{\text{max}}$ ), PCA axes, leaf and root traits. Significant values (p-value <0.05) are indicated in bold.

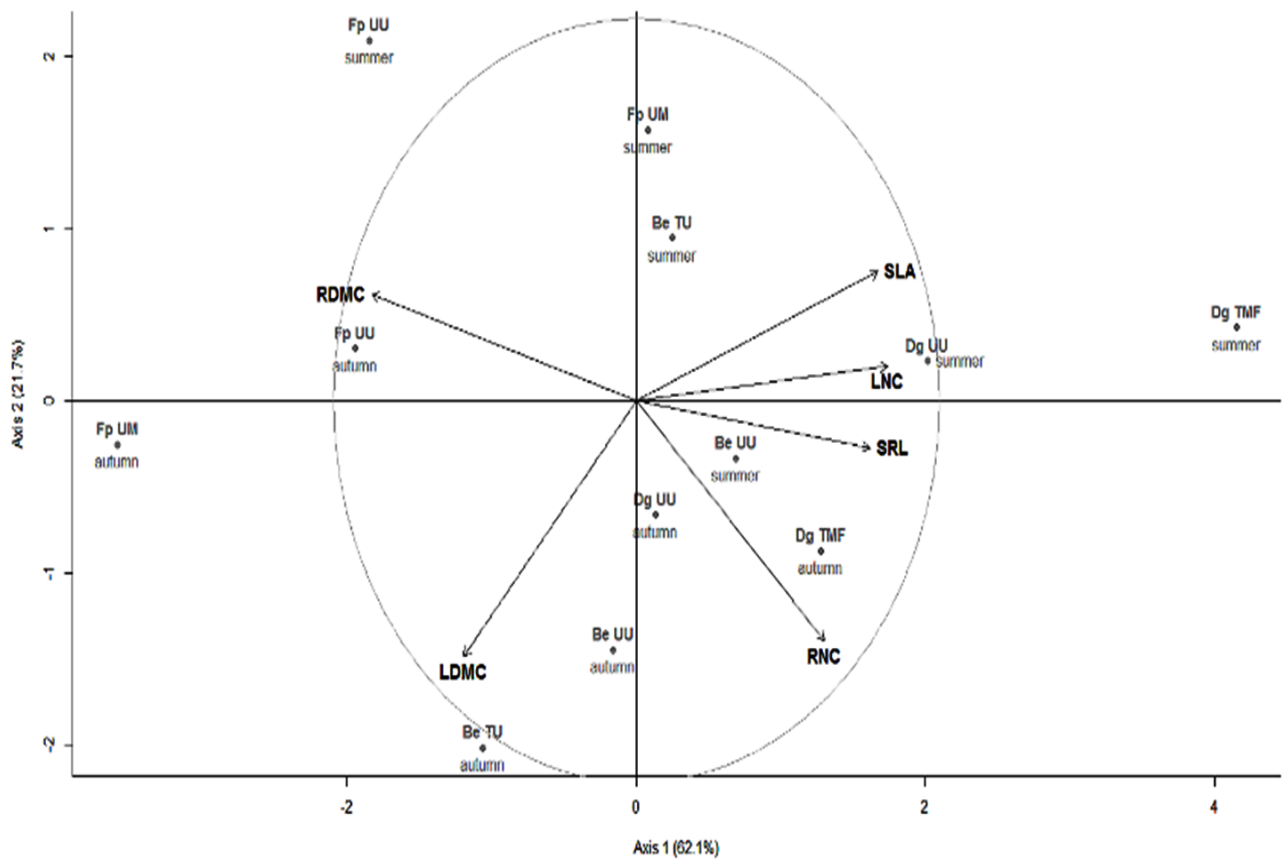
	$V_{\text{max}}\text{NH}_4^+$	$V_{\text{max}}\text{NO}_3^-$	axe1	axe2	ratio	SLA	LDMC	SRL	RDMC	LNC
$V_{\text{max}}\text{NO}_3^-$	<b>0.93</b>									
axe1	<b>0.65</b>	<b>0.76</b>								
axe2	-0.28	-0.24	0.00							
ratio	-0.46	<b>-0.68</b>	<b>-0.64</b>	0.43						
SLA	0.37	0.53	<b>0.84</b>	0.38	-0.54					
LDMC	-0.17	-0.26	<b>-0.60</b>	<b>-0.74</b>	0.09	<b>-0.74</b>				
SRL	0.55	<b>0.70</b>	<b>0.81</b>	-0.14	-0.51	0.54	-0.34			
RDMC	<b>-0.68</b>	<b>-0.77</b>	<b>-0.91</b>	0.31	<b>0.75</b>	<b>-0.63</b>	0.37	<b>-0.71</b>		
LNC	<b>0.64</b>	<b>0.65</b>	<b>0.87</b>	0.10	-0.32	<b>0.69</b>	-0.51	<b>0.66</b>	<b>-0.74</b>	
RNC	<b>0.58</b>	<b>0.62</b>	<b>0.65</b>	<b>-0.70</b>	<b>-0.80</b>	0.34	0.08	0.50	<b>-0.83</b>	0.42

**Table 4.** Soil properties (mean values  $\pm$  SE) for each grassland and at each sampling time. No significant difference values between sites at a given date are shown by the same letter (Tukey post-hoc test). Values in bold indicate the highest values when the considered soil parameter was significantly different between dates in a grassland. na: not available because of a sampling issue. nd: not detectable: under the level of sensitivity of the method; TDN: total dissolved nitrogen).

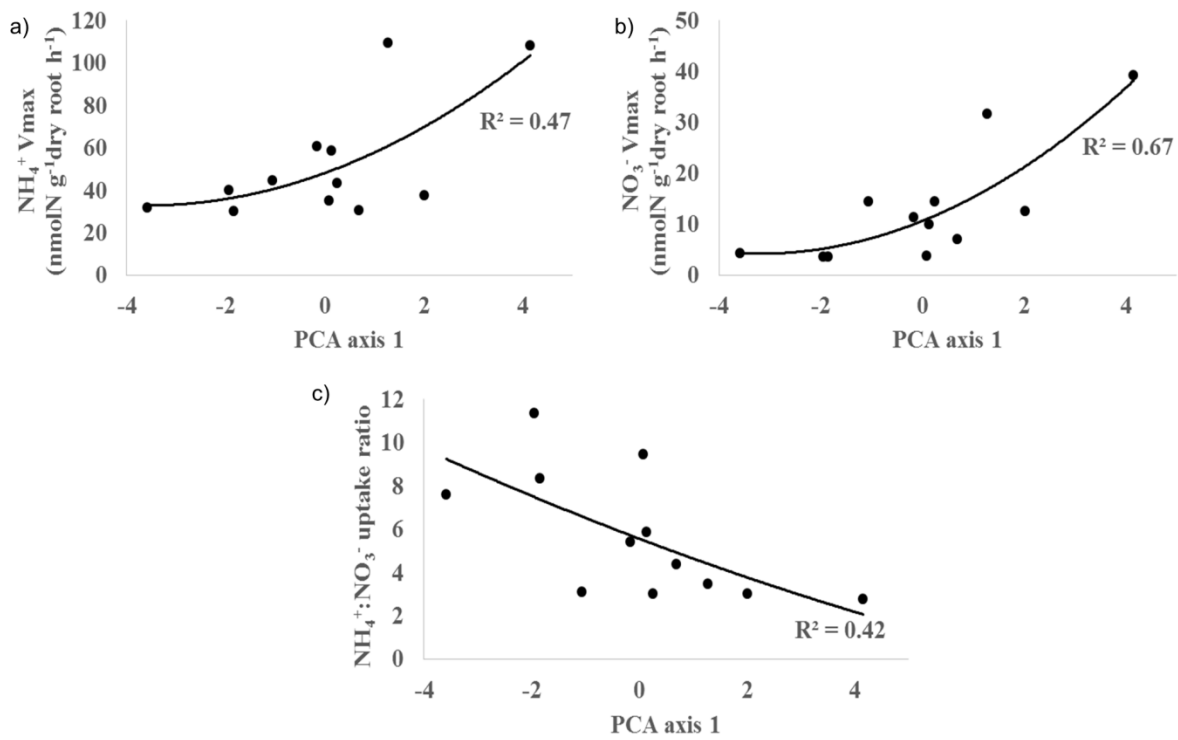
		TMF	TU	UM	UU
Soil Water	Summer	<b>22.33 <math>\pm</math> 0.71<sup>b</sup></b>	<b>18.33 <math>\pm</math> 1.12<sup>c</sup></b>	na	<b>34.89 <math>\pm</math> 1.20<sup>a</sup></b>
Content (%)	Autumn	11.44 $\pm$ 0.33 <sup>b</sup>	7.25 $\pm$ 0.88 <sup>c</sup>	13.58 $\pm$ 1.18 <sup>b</sup>	17.74 $\pm$ 1.54 <sup>a</sup>
pH	Summer	8.01 $\pm$ 0.04 <sup>a</sup>	8.03 $\pm$ 0.04 <sup>a</sup>	n.a.	<b>6.31 <math>\pm</math> 0.05<sup>b</sup></b>
	Autumn	7.98 $\pm$ 0.02 <sup>a</sup>	8.05 $\pm$ 0.04 <sup>a</sup>	5.85 $\pm$ 0.05 <sup>b</sup>	6.02 $\pm$ 0.08 <sup>b</sup>
Soil Organic	Summer	13.16 $\pm$ 0.48 <sup>c</sup>	14.39 $\pm$ 0.93 <sup>b</sup>	n.a.	18.42 $\pm$ 0.64 <sup>a</sup>
Matter (%)	Autumn	12.47 $\pm$ 0.65 <sup>b</sup>	11.38 $\pm$ 0.93 <sup>b</sup>	14.02 $\pm$ 0.43 <sup>ab</sup>	16.98 $\pm$ 1.29 <sup>a</sup>
C:N ratio	Summer	<b>14.91 <math>\pm</math> 0.35<sup>a</sup></b>	14.02 $\pm$ 0.64 <sup>a</sup>	n.a.	12.10 $\pm$ 0.15 <sup>b</sup>
	Autumn	13.46 $\pm$ 0.39 <sup>a</sup>	13.43 $\pm$ 0.64 <sup>ab</sup>	11.63 $\pm$ 0.14 <sup>b</sup>	11.74 $\pm$ 0.43 <sup>b</sup>
TDN ( $\mu\text{gN}\cdot\text{g}^{-1}$ soil)	Summer	20.83 $\pm$ 2.95 <sup>b</sup>	16.85 $\pm$ 1.33 <sup>b</sup>	n.a.	55.59 $\pm$ 12.15 <sup>a</sup>
	Autumn	<b>46.12 <math>\pm</math> 4.44<sup>a</sup></b>	<b>46.13 <math>\pm</math> 4.74<sup>a</sup></b>	34.54 $\pm$ 1.21 <sup>ab</sup>	46.65 $\pm$ 6.04 <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> content ( $\mu\text{gN}\cdot\text{g}^{-1}$ soil)	Summer	3.87 $\pm$ 0.48 <sup>ab</sup>	<b>5.63 <math>\pm</math> 0.64<sup>a</sup></b>	n.a.	2.31 $\pm$ 0.53 <sup>b</sup>
	Autumn	2.99 $\pm$ 0.54 <sup>a</sup>	0.54 $\pm$ 0.10 <sup>b</sup>	n.d.	1.39 $\pm$ 0.66 <sup>ab</sup>
NH <sub>4</sub> <sup>+</sup> content ( $\mu\text{gN}\cdot\text{g}^{-1}$ soil)	Summer	11.19 $\pm$ 0.47 <sup>a</sup>	15.43 $\pm$ 1.82 <sup>a</sup>	n.a.	12.23 $\pm$ 1.77 <sup>a</sup>
	Autumn	10.07 $\pm$ 1.37 <sup>a</sup>	11.54 $\pm$ 1.55 <sup>a</sup>	6.97 $\pm$ 0.94 <sup>a</sup>	10.17 $\pm$ 1.92 <sup>a</sup>
NH <sub>4</sub> <sup>+</sup> :NO <sub>3</sub> <sup>-</sup> ratio	Summer	3.56 $\pm$ 0.65 <sup>b</sup>	2.97 $\pm$ 0.30 <sup>b</sup>	n.a.	7.85 $\pm$ 1.62 <sup>a</sup>
	Autumn	3.53 $\pm$ 0.36 <sup>b</sup>	<b>28.68 <math>\pm</math> 10.7<sup>a</sup></b>	n.d.	12.9 $\pm$ 6.4 <sup>ab</sup>

Figures legends:

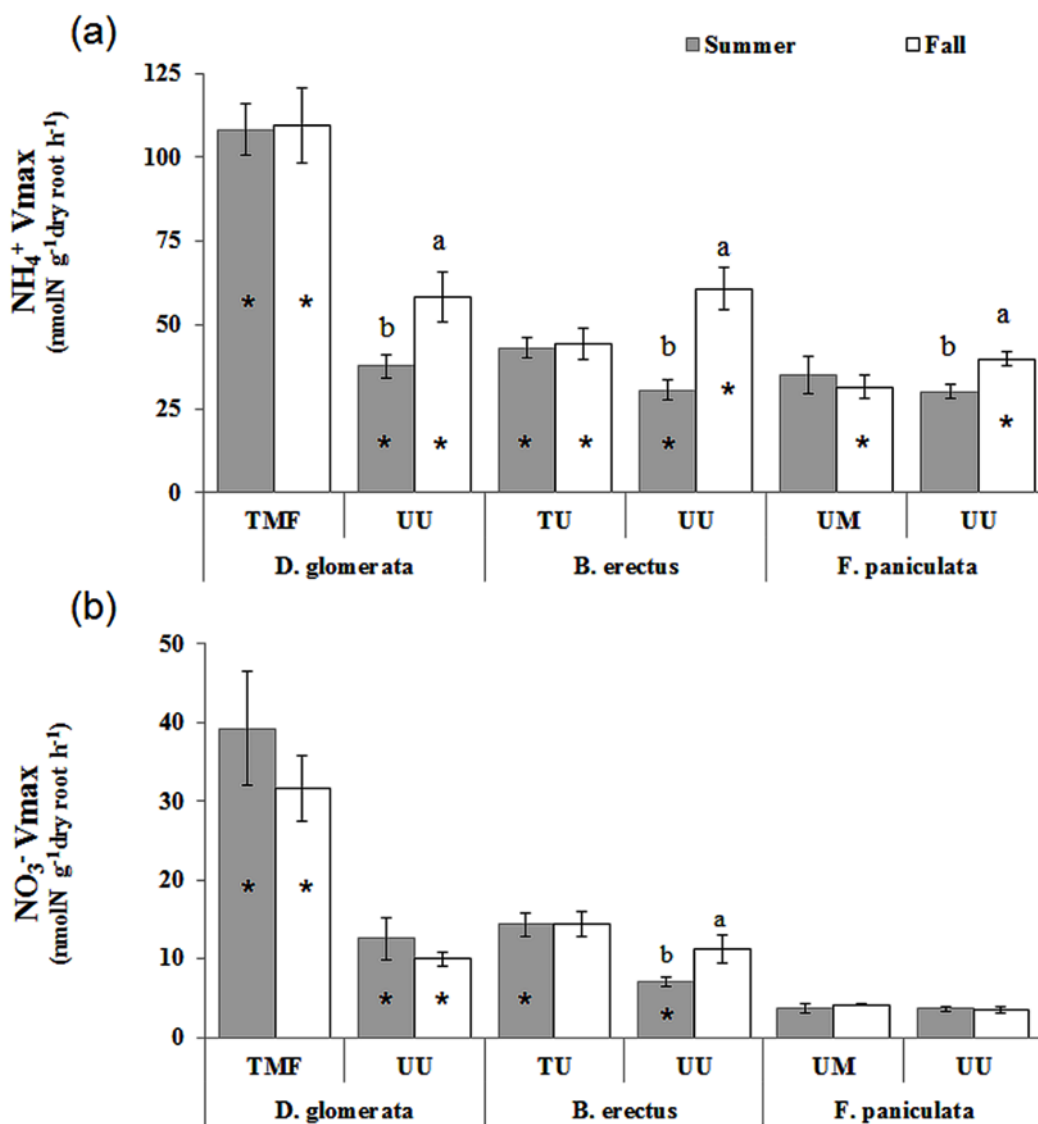
**Figure 1.** Principal components analysis (PCA) of functional traits measured for the leaves and roots of three grass species (Be: *Bromus erectus*, Dg: *Dactylis glomerata* and Fp: *Festuca paniculata*), in each grassland with different management (UU: unterraced unmown, UM: unterraced mown, TMF: terraced mown and fertilized, TU: terraced unmown). SLA: Specific leaf area, LDMC: Leaf dry matter content, LNC: Leaf nitrogen content, SRL: Specific root length, RDMC: Root dry matter content, RNC: Root nitrogen content.



**Figure 2:** Relationships between the first axes of the PCA (fig 1) and Vmax for  $\text{NH}_4^+$  (a),  $\text{NO}_3^-$  (b) and  $\text{NH}_4^+:\text{NO}_3^-$  uptake ratio (c). The three relationships were significant (p-values<0.05) assuming a polynomial relationship of order=2, and the resulting  $R^2$  are indicated on each graph.



**Figure 3.**  $V_{max}$  (Maximal uptake rate) for  $\text{NH}_4^+$  (a) and  $\text{NO}_3^-$  (b) of *D. glomerata*, *B. erectus* and *F. paniculata*. Within each combination of site and species, dates with the same letter had similar uptake parameters (Tukey post hoc test at 5% level, after an Anova with date as main effect). For each species, the significance of the differences between the two sites for uptake parameters were tested using a Student test, and stars indicate the dates at which the two sites differ significantly with a  $p$ -value < 0.05.



**Figure 4:**  $\text{NH}_4^+:\text{NO}_3^-$  uptake ratio for the three species, in each site and at the two sampling times. The uptake ratio is unitless (ratio between  $\text{NH}_4^+V_{\text{max}}$  and  $\text{NO}_3^-V_{\text{max}}$ ). Within each species, \*or \*\*\* indicate significant site effects ( $p$  value  $< 0.05$  and  $0.001$  respectively) within each species (two-ways ANOVA with site, date and the interaction as main effect). In the grassland (UU) where all species occurred, the differences between species and sampling time were tested using two-ways ANOVA. Similar letters connect species with similar values in the UU grassland at both sampling dates.

