Studies of NH_4^+ and NO_3^- uptake ability of subalpine plants and resource-use strategy identified by their functional traits.

Grassein F. [1,2]., Legay N.[3,4,5], Arnoldi C. [3], Segura. R.[1,2], Laîné P.[1,2], Lavorel S.[3], Clément J.C.[3,6].

[1] Université de Caen Basse-Normandie, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, CS 14032, 14032 CAEN Cedex 5, France

[2] INRA, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, CS 14032, 14032 CAEN Cedex 5, France

[3] Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France

[4] Ecole de la Nature et du Paysage, INSA Centre Val de Loire, 9 Rue Chocolaterie, 41000 Blois, France

[5] CNRS, CITERES, UMR 7324, 37200 Tours, France

[6] CARRTEL, UMR 0042 INRA-Univ. Savoie Mont Blanc, FR-73376, Le Bourget du Lac, France

Running title: plant N uptake and functional strategy

22   SUMMARY:

23   The leaf economics spectrum (LES) is based on a suite of leaf traits related to plant

24   functioning and ranges from resource-conservative to resource-acquisitive strategies.

25   However, the relationships with root traits, and the associated belowground plant

26   functioning such as N uptake is still poorly known. Additionally, environmental variations

27   occurring both in time and in space could uncouple LES from root traits. We explored, in

28   subalpine grasslands, the relationships between leaf and root morphological traits and to

29   what extent they contribute to the plant resource economics spectrum (RES). We also

30   investigated the link between this spectrum and N uptake, as well as the variations of N

31   uptake across different grasslands and during the growing season. Although poorly

32   correlated with leaf traits, root traits contributed to a RES at the whole plant level. Higher

33   NH_4^+ and NO_3^- uptake abilities were associated with the resource-acquisitive strategy.

34   Nonetheless, N uptake within species varied between grasslands and during the growing

35   season, suggesting that LES and plant traits are good, but still incomplete, descriptors of

36   plant functioning. Soil environmental variations between grasslands or during the growing

37   season may also drive to some extent the N uptake ability of species. Our results support

38   the current efforts to build a more general framework including above- and below-ground

39   processes when studying plant community functioning.

40   Keywords: Leaf and root traits, Nitrate and ammonium uptake, plant assimilation, Resource

41   use strategy, Subalpine grasslands

42   Introduction

43   Functional traits have been widely used to describe different plant strategies. One major axis

44   of specialisation involves a trade-off between conservation of resources in well protected and

45   long lived tissues, and acquisition of resources by tissue with high use-efficiency and turn-

46   over, and commonly referred as the leaf economic spectrum (LES, Wright *et al*. 2004). More

47   specifically, species with an exploitative strategy share similar leaf attributes such as high

48   specific leaf area (SLA) and nitrogen concentrations (LNC) that have been associated with

49   short leaf life-span, high photosynthetic capacity (PNUE) as well as high decomposability

50   (Reich *et al.* 1998, Cornwell *et al.* 2008). And the conservation/acquisition strategies have

51   been numerously linked to plant ecology, fast-growing exploitative species dominating in

52   nutrient rich environment while the slow-growing conservative species are more common in

53   poor or harsh conditions (Chapin 1980, Lambers & Poorter 1992, Ordonez et al. 2009).

54   Despite some evidences of a similar contribution of root traits to the plant strategy (Roumet et

55   al. 2006, Freschet et al. 2010, Fort et al. 2013), the importance of root traits is less understood

56   than for leaf traits, mainly because weak correlations between analogous leaf and root traits

57   have been reported (Craine et al 2005, Tjoelker et al. 2005, Freschet et al. 2010), and also

58   because root functioning is often overlooked compared to leaves in field conditions.

59   Nutrient uptake ability is one of the main functions provided by roots, and both physiological

60   and morphological adjustments are involved at the root level to support changes in nutrient

61   shoot demand (Chapin 1980, Hodge 2004, James et al. 2009). Nutrient uptake is indeed

62   influenced both by anatomical properties of roots involved in the prospection such as specific

63   root length, as well as physiological adjustments such as kinetic parameters like maximal

64   uptake rate (Vmax, but see Bassirirad 2000). Among nutrients, nitrogen is one of the most

65   well studied one, especially its inorganic forms (NO_3^- and NH_4^+) both for plants and for

66   ecosystem functioning. However, rarely have morphological and physiological properties of

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67   root been assessed simultaneously in field conditions, whereas some supports come from

68   species grown in standardized conditions (Maire et al. 2009, Grassein et al. 2015). Nitrogen

69   uptake can indeed be influenced by several environmental factors justifying the use of

70   controlled conditions to estimate uptake parameters in a comparative purpose. For example,

71   nitrogen uptake has been reported to vary in response to temperature or pH (Garnett and

72   Smethurst 1999). Nevertheless, N uptake ability also differs between species, and is partially

73   related to plant strategy and their functional traits (Grassein et al. 2015), but these results need

74   to be validated for plant grown in natural conditions.

75   Subalpine grasslands are subject to the combined effects of climate and anthropogenic factors,

76   both influencing N cycling and thus N availability for organisms (Bardgett *et al.* 2005, Legay

77   *et al.* 2013). Decreased management intensity favours plant species with resource

78   conservative traits (Quétier *et al.* 2007), which are usually associated with fungal-dominated

79   belowground communities (de Vries *et al.* 2012, Grigulis *et al.* 2013). Concomitantly, it slows

80   down N cycling (Zeller *et al.* 2000, Robson *et al.* 2010), favouring the accumulation of soil

81   ammonium (NH_4^+) rather than soil nitrate (NO_3^-) (Robson *et al.* 2007). Plants growing in such

82   variable conditions are likely to adjust their N uptake ability, as it has been shown for

83   functional traits (Quétier *et al.* 2007, Grassein et al. 2010). Although measuring only a net N

84   uptake, which is the result of influx and efflux, the direct measurement of N uptake using

85   excised roots allows to characterise the uptake kinetics for NO_3^- and NH_4^+ of several species

86   while controlling the environmental variations and thus making it possible to compare

87   different species at the cost to lose some of ecological meanings (Lucash et al. 2007). This

88   method could thus be applied to plants collected in the field in order to complement previous

89   *in situ* field studies in the investigation on the links between functional traits (leaf and roots)

90   and N uptake ability for species where environmental constraints were free to influence plant

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91   performance and likely to differ between aboveground and belowground organs (Craine et al.

92   2005).

93   In this study, we investigated the relationships between functional traits and inorganic N

94   uptake for three perennial grass species with contrasted LES. Because soil inorganic N

95   availability and plant N uptake ability are likely to vary across seasons and in response to

96   management (Jaeger *et al.* 1999, Miller *et al.* 2009), we examined these relationships for

97   individuals occurring in grasslands with different management and throughout the growing

98   season, thereby testing their temporal consistency. Using the excised roots method for root N

99   uptake estimation, and measuring functional traits for leaves and roots, we tested the

100   following hypotheses: (1) the root traits are also contributing to the economics spectrum

101   observed for leaf traits, (2) and more exploitative species should be more efficient to take up

102   N. As functional traits are influenced by environmental conditions, we hypothesise that N

103   uptake will be influenced by environmental variations (3) between grasslands, as well as

104   during the growing season, probably following N availability, both in quantity and in quality

105   depending on the most abundant form.

106

107   Material and methods

108   Study site and species

109   The study site is located in the upper Romanche valley of the central French Alps between the

110   village of Villar d'Arêne and the Lautaret Pass. The climate is subalpine with a strong

111   continental influence. Winters are cold and snowy, with monthly average minimum

112   temperatures of -15.9°C in February, maximum monthly average temperature of 23.8°C in

113   July, and mean annual precipitation of 956mm (unpublished data, sajf.ujf-grenoble.fr). The

114   growing season starts following snow melt in late April - early May and continues until late

115   September or October depending on the date of the first snow in autumn.

116   Given the hypothesis that NH_4^+ and NO_3^- uptake could be an important hard plant trait related

117   to resource use strategy (as suggested by soft structural and morphological traits) and to field

118   dominance, and due to the degree of precision chosen for N uptake estimations (see 2.2), a

119   compromise was necessary regarding the number of species, grasslands and replicates to be

120   investigated. This sampling adjustment was required to conduct N uptake estimations for all

121   individuals in a brief enough time period so that most abiotic factors remained as comparable

122   as possible (soil moisture, temperature, radiation).

123   We chose three common and dominant grass species, *Dactylis glomerata* L., *Bromus erectus*

124   Huds and *Festuca paniculata* Schinz and Tell. All species are perennial, arbuscular

125   mycorrhizal non-dependent species and span a gradient from more exploitative (*D.*

126   *glomerata*) to more conservative (*F. paniculata*) resource use strategies (Grassein et al. in

127   press). Four grasslands (Table 1), described in Quétier *et al.* (2007), were chosen for their

128   contrasting past and current management, and were similar to the grasslands studied by

129   Robson *et al.* (2007, 2010) : (i) Terrace Mown and Fertilized (TMF), (ii) Terrace Unmown

130   not fertilized but lightly grazed (TU), (iii) Un-terraced Mown grassland (UM) and (iv) Un-

131   terraced Unmown but lightly grazed grassland (UU), representing a gradient of decreasing

132   management intensity. To reflect field dominance patterns, *D. glomerata* was sampled in

133   TMF, *B. erectus* in TU, *F. paniculata* in UM, and all three species were sampled in UU where

134   they coexist, although *F. paniculata* was dominant (Table 1).

135   To assess N uptake patterns over the growing season, the same sampling design was repeated

136   twice during 2010. At each date for each species and grassland, we sampled the roots and soil

137   (approximately: 25x25x25 cm) of five individuals (genetically distinct individuals at least 2m

138   apart). The first sampling corresponded to the peak biomass and targeted flowering onset (just

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139   before anthesis), and the second sampling corresponded to autumn after last management

140   activities occurred. For *D. glomerata* in TMF and *B. erectus* in TU, the two sampling dates

141   were mid-June and mid-September. For *F. paniculata* in UM and the three species in UU, the

142   sampling dates were: early July and early September. These two dates are called "Summer"

143   and "Autumn" hereafter. As much as possible, species were sampled at the same time the day

144   to avoid any diurnal variation in N uptake (Gessler et al. 1998).

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146   Soil nitrogen pools

147   At each date and for each grassland, soil nitrogen concentrations were measured from six soil

148   cores (dimensions 4.5 cm Ø, 10 cm deep) kept on ice in the field and maintained at 4°C upon

149   return to the laboratory (within 2h). Soils were sieved through a 5.6-mm mesh to remove roots

150   and stones. A subsample of 10g fresh sieved soil was prepared for extraction of inorganic N in

151   0.5M K_2SO_4, and analysed using a colorimetric analyser (FS-IV autoanalyser (OI-Analytical,

152   College Station, TX, USA) (following Bowman *et al*. 2003) to measure soil concentrations of

153   ammonium (NH_4^+), nitrate (NO_3^-) and Total Dissolved Nitrogen (TDN). Soil aliquots were

154   used to determine soil water (7 days at 70°C) and soil organic matter contents (550°C during

155   4 hours). Finally, soil subsamples were air-dried to measure soil pH, or ground to a fine

156   powder for measurements of total carbon (C) and N contents using an elemental analyser

157   (FlashEA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA).

158   At each date, five individuals of each species, with roots and soil, were excavated from each

159   field and transferred within half an hour to the laboratory located at the Lautaret Pass (Station

160   Alpine Joseph Fourier). Living and young fine roots were washed with deionised water, cut to

161   2-cm length and stored in 1mM $CaSO_4$ for less than two hours to limit the uptake decline

162   starting around three hours after excision (Louahlia *et al.* 2000). Root samples from each

163   individual were separated into 14 sub-samples of approximately 0.5g fresh mass.

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165   Functional traits

166   Functional traits were measured for roots and leaves using standardised protocols

167   (Cornelissen *et al.* 2003). Two of the individual root sub-samples were used to estimate root

168   dry matter content (RDMC), specific root length (SRL, Winrhizo® software, fresh length per

169   unit of dry mass), and were further analysed to obtain root-^{15}N natural abundance and root

170   nitrogen concentration (RNC, N mass per unit of dry mass). Specific leaf area (SLA, fresh

171   area per unit of dry mass), leaf and root dry matter contents (LDMC and RDMC, dry mass per

172   unit of fresh mass), leaf nitrogen concentration (LNC, N mass per unit of dry mass) and

173   specific root length (SRL, Winrhizo® software, fresh length per unit of dry mass) were also

174   measured.

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176   Nitrogen uptake estimation: the "excised" roots method

177   Root N uptake kinetics started within 60 min after excision, thereby avoiding the potential

178   decline in N uptake ability reported to start after 3h (Louahlia et al. 2000). Nitrate and

179   ammonium uptake by plants involved mainly the transport system called HATS (High

180   Affinity Transport System). It contributes to N uptake at low to moderate concentrations of

181   external N (<1mM) and saturates at 0.2-0.5 mM (Kronzucker *et al.* 2000, Min *et al.* 2000),

182   which makes it the more likely system used by plants growing in natural and semi-natural

183   ecosystems limited by N (Bassirirad 2000, Maire *et al.* 2009). The estimation of the maximum

184   NH_4^+ and NO_3^- uptake rates by HATS requires a range of N concentrations below 1mM at

185   which the Vmax can be reached depending on species (Grassein et al. 2015). Consequently,

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186   uptake was estimated from the accumulation of ^{15}N in root sub-samples incubated for one

187   hour in a buffer solution (pH = 5.5- following Leon *et al.* (1995)), containing a range of N

188   concentrations (20, 50, 100, 250, 500 and 1000 µM). Six sub-samples were incubated in

189   K^{15}NO$_3$ and the other six in (^{15}NH$_4$)$_2$SO$_4$ with a ^{15}N excess of 99% atom. The two N forms

190   were tested individually in order to avoid possible interactions (Kronzucker et al. 1999)

191   Solution volumes and fresh weights were selected to avoid N depletion during the experiment.

192   After 1h incubation, roots were washed twice for one minute with a 1mM CaSO$_4$ at 4°C to

193   stop any metabolic processes. Roots were then dried at 60°C for 72h, ground to a fine powder

194   and analysed by IRMS at the University of Caen (Isoprime GV instruments, Stockport, UK)

195   to obtain ^{15}N Atom% and N concentrations.

196

197   Data analysis

198   Nitrogen Uptake Rate (NUR) was calculated for each concentration and each inorganic N

199   form (NH$_4^+$ and NO$_3^-$) using the ^{15}N increase in the root incubated compared to the non-

200   incubated control, and expressed by unit of time and dry mass (nmolN.h^{-1}.g^{-1} of dry roots, see

201   Leon *et al.* 1995). The dependence of NUR on substrate concentration was fitted for each

202   individual and Hanes's relations (Michaelis transformation) was used to estimate the

203   maximum uptake rate (Vmax) defined as the maximum NUR for NH$_4^+$ and NO$_3^-$ (Leon *et al.*

204   1995). Finally, the NH$_4^+$:NO$_3^-$ uptake ratio was calculated as the ratio between NH$_4^+$ Vmax

205   and NO$_3^-$ Vmax.

206   A principal component analysis (PCA) was performed using all plant functional traits at the

207   individual level to describe their functional strategy based on leaf and root traits.

208   Relationships between functional traits of leaves and roots, and uptake ability were

209   investigated using Pearson correlation coefficients. Relationships between the functional

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210   strategy and N uptake at the root level were tested using regression analyses between the N

211   uptake parameters (Vmax and Km) and the first PCA.

212   Comparisons of uptake parameters for species, fields and date were conducted with ANOVA

213   followed by Tukey tests to compare species and grasslands. Similarly, we used ANOVA and

214   Tukey post hoc test to test soil parameters differences between grasslands and dates. Data

215   were log-transformed when necessary to achieve normality and heteroscedasticity.

216   All statistical analyses were performed using the software R 2.14.0 (R Core team

217   2012), with multivariate analyses (PCA) being performed using the package Ade4 (Dray &

218   Dufour 2007).

219

220   Results

221   We observed large variations for leaf and root functional traits in spite of a restricted number

222   of species in our study (Table 2). The range of variation was similar to, and sometimes even

223   higher than the variability reported in Fort *et al.* (2013) for a larger set of species occurring in

224   a similar ecosystem, including *D. glomerata* and *B. erectus*. The PCA of functional traits

225   highlighted a first axis explaining 62.1% of the total variance (Fig. 1). The three species

226   differed significantly for their mean position along this axis (p=0.012), with positive values

227   for *D. glomerata* and negative values for *F. paniculata*. Positive values along this axis were

228   characterised by high SLA, LNC and SRL, and low LDMC. Among these, SLA and LNC

229   have been reported as major contributors to a resource economic spectrum establishing the

230   existence of a fundamental trade-off between plant features allowing resource capture and

231   those allowing resource conservation.

232   This functional axis was positively correlated to NH_4^+ and NO_3^- Vmax (Fig 2a and 2b) and

233   negatively to $NH_4^+:NO_3^-$ uptake ratio (Fig 2c) indicating a more pronounced preference for

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234   NH_4^+ at lower values of axis 1. Except RDMC, all traits taken separately were poorer

235   predictors of the NO_3^- and NH_4^+ maximum uptake rates than this functional axis, although the

236   first PCA axis was significantly correlated with all functional traits (Table 3).

237   In UU grassland, NH_4^+Vmax in summer was similar for the three species (Fig. 3a) but greater

238   for *D. glomerata* for NO_3^- Vmax (p <0.001, Fig. 3b). Vmax in autumn for both N forms was

239   lower for *F. paniculata* compared to the two other species (NH_4^+ p<0.05, NO_3^- p< 0.001).

240   Comparing the different grasslands within species, we observed reduced NO_3^- and NH_4^+

241   Vmax values in the UU grassland for *D. glomerata* (in summer and in autumn) and *B. erectus*

242   (summer) compared to the other grasslands. On the other hand, highest NH_4^+ Vmax for *B.*

243   *erectus* and *F. paniculata* were found in UU during the autumn. Illustrating the seasonal

244   variability, all species in the UU grassland had higher NH_4^+ maximum uptake rates in the

245   autumn than in the summer, as well as higher NO_3^- uptake for *B. erectus*. NH_4^+:NO_3^- uptake

246   ratio did not vary in time, but always showed higher values in the UU for the three species

247   compared to the other grasslands, and overall greater values for *F. paniculata* (Fig. 4).

248   Within species, a limited number of traits were significantly different between grasslands

249   (Table 2). We only observed significant differences in autumn, with highest LDMC in TU for

250   *B. erectus*, highest LDMC and RDMC in UU for *D. glomerata*, and highest LDMC but lowest

251   SRL for *F. paniculata* in UM. However, changes in response to the season were more

252   consistent among species and grasslands, with an increase of LDMC and a decrease of SLA in

253   autumn compared to the summer. We also observed higher LNC for *D. glomerata* and *F.*

254   *paniculata* during the summer than during the autumn in TMF and UM respectively, and

255   higher RDMC during the autumn for *D. glomerata* in UU.

256   Since all species occurred in the UU grasslands, we choose to focus on soil parameters from

257   this grassland. UU and UM only differ for SWC in autumn (Table 4), all other soil variables

258   were similar between these two grasslands, which had similar past land-use history (Table1).

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259 UU had consistently higher SWC and SOM, and lower soil pH and CN ratio than TMF and

260 TU. All grasslands had similar soil NH_4^+ concentrations. During the summer, we observed

261 higher TDN and NH_4^+:NO_3^- soil ratio, and lower soil NO_3^- concentration in UU compared to

262 TMF and TU, but we did not find these differences in autumn.

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264 Discussion:

265 Relationships between leaf and root traits

266 In the aim to find parallels between above and below-ground organs (e.g. Roumet et al. 2006),

267 several studies have investigated the relationships between analogous traits measured for

268 leaves and roots. While positive relationships have been reported for SLA *vs* SRL (Craine &

269 Lee 2003, Craine et al. 2005, Freschet et al. 2010), other studies have reported a lack of

270 relationships between SLA *vs* SRL (Craine et al. 2001, Tjoelker et al. 2005). Consequently,

271 the absence of relationships in our study between SRL/SLA, LDMC/RDMC and LNC/RNC

272 was not unexpected, although our limited number of species/replicates could explain a

273 reduced number of significant relationships.

274 Nevertheless, we observed similar trade-offs at the leaf and root levels between traits, namely

275 N concentration and dry matter content. A similar spectra of resource use at the leaf and root

276 levels has already been reported (Freschet et al. 2010), despite a relatively low degree of

277 relationships between analogous traits belowground and aboveground. Different selective

278 pressure for leaf and root traits as well as specialisation for the acquisition of different

279 resources (e.g. light *vs* nutrient) could explain this absence of association between

280 belowground and aboveground traits (Craine et al. 2005, Liu et al. 2010), while the global

281 strategy at the plant level could remain the same since high efficiency for light or for nutrients

282 could be related to the same physiological adaptation, as pointed out previously for stress

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283   tolerance (Chapin 1980). However, leaf functional traits had been shown to have a limited

284   power to predict N uptake at the root level (Craine & Lee 2003), and root traits remain more

285   informative for this purpose.

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289   Relationship between N maximum uptake rate (Vmax) and plant strategy

290   Our results showed that a stronger exploitative syndrome (higher SRL, SLA, LNC and lower

291   RDMC) was associated with higher Vmax for both inorganic N forms, rejecting the

292   hypothesis of a trade-off between maximum uptake rate of each N forms (ref ou). Indeed,

293   ammonium toxicity has been reported for some plant species (review in Britto & Kronzucker

294   2002), as well as negative interactions between the uptake of NH_4^+ and NO_3^- (Kronzucker et

295   al. 1999), and this could promote a trade-off in the acquisition of NH_4^+ and NO_3^- between

296   species (Maire et al. 2009). Here, we estimated NH_4^+ and NO_3^- uptake independently to avoid

297   such interactions during measurements, and our results did not support a trade-off but rather

298   suggest a synergistic uptake of both N forms. Provision of NO_3^- has been demonstrated to

299   alleviate the NH_4^+ toxicity (Britto & Kronzucker 2002), and even to favour NH_4^+ uptake. We

300   indeed observed higher uptakes for NH_4^+ than for NO_3^-, indicating a preference of all species

301   for NH_4^+, especially for individuals with a more conservative syndrome of traits. Besides,

302   more exploitative plants have a lower preference for NH_4^+ compared to more conservative

303   individuals, but expressed higher maximal uptake rates than more conservative individuals for

304   both N forms.

305   Overall, changes in functional leaf traits related to a higher potential photosynthesis efficiency

306   and light capture appeared to be associated at the root level with higher N maximal uptake

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307   rates for both N forms, eventhough leaf traits alone appeared as a poor predictors of N uptake

308   (Craine & Lee 2003).

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310   Nitrogen uptake variations in response to management and sampling dates

311   Nitrogen uptake rate is usually considered as a property of plant species, but little is

312   known about variation in within-species N uptake rates in grasslands with different land-use

313   history and at different times during the growing season. In our study, we observed that

314   nitrogen uptake rates could differ strongly for the same species in different grasslands (e.g. *B.*

315   *erectus* and *D. glomerata* in the UU grassland). On the other hand, the time of the year also

316   influenced the N uptake rates of all species, with for example an opposite response between

317   grasslands for *B. erectus* depending on the sampling date. Overall, grasslands were weakly

318   discriminated by functional traits, suggesting that other factors such as soil parameters may

319   explain the within species N uptake differences between grasslands.

320   Nitrogen uptake can vary depending on the amount of N available in the soil (Gavito

321   et al. 2001). Soil NH_4^+ concentration, the main N source taken up by plants in our study, was

322   similar in the four investigated grasslands, whereas a higher soil TDN was measured in the

323   UU grassland. Consequently, the lower N uptake rates observed in this grassland cannot be

324   explained by a lower N availability. As reported by previous studies, subalpine grasslands can

325   show the legacy effects of former management activities, leading to slower N cycling (Zeller

326   et al. 2000, Robson et al. 2007). Indeed, we observed lower pH and higher soil water and

327   organic matter contents in the UU grassland suggesting variations in N cycling and in the

328   quality of the available N, not only in its quantity (Garnett & Smethurst 1999, Robson et al.

329   2010). Supporting this hypothesis, we observed variations in soil NO_3^- concentrations, and

330   consequently soil $NH_4^+:NO_3^-$ ratio, between the studied grasslands. Although we could not

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331   directly relate *in situ* soil parameters to N plant uptake estimated under "controlled"

332   conditions, we interestingly observed parallel changes for NH_4^+ uptake and soil NH_4^+: NO_3^-

333   ratio in grasslands where individuals have been sampled. For example, both *B. erectus* NH_4^+

334   uptake and soil NO_3^- concentration were lower during the summer and higher during the

335   autumn in TU than in UU.

336   Rarely investigated in natural ecosystems, experimental evidences on cultivated plants

337   have demonstrated the effects of soil NH_4^+: NO_3^- ratio concentration on plant N uptake

338   (Errebhi & Wilcox 1990, Bar-Tal et al. 2001). Yet, the effects were largely species-dependent

339   and trade off were sometime reported between NH_4^+ and NO_3^- uptakes (Warncke & Barber

340   1973, Kronzucker et al. 1999, Maire et al. 2009). The preferential uptake for an inorganic N

341   form could also be influenced by environmental and physiological factors (Britto &

342   Kronzucker 2013). Our results did not support any trade-off in the intrinsic ability of plant

343   species to take up both N forms, even after removing possible environmental conditions or

344   interactions between N forms. Although we could not directly test for the relationship

345   between soil parameters and plant N uptake rates, differences between grasslands in the N

346   uptake within species highlight that management practices may have important effects on

347   plant N uptake, likely through N cycling changes and the quality of N pool available as alreay

348   pointed out by previous studies (Zeller et al. 2000, Robson et al. 2007). Other studies have

349   suggested that N preference could be dependent of the soil availability of the different N

350   forms (Näsholm et al. 2009, Stoelken et al. 2010). While our results partially supported this

351   hypothesis, with variation within species between different grassland, the different species

352   sampled in the same grassland showed differences in their NH_4^+:NO_3^- uptake ratio, supporting

353   the hypothesis that this "preference" is partially related to the strategy of species, or at least to

354   species identity. But overall, more exploitative species with higher maximum uptake rates for

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355   one N form is also likely to have high uptake rates for other N forms (Kastovska &

356   Santruckova 2011).

357   Nevertheless, the plant preference for N forms is a complex topic (Britto &

358   Kronzucker 2013), and careful considerations should be given to the environmental

359   conditions where the species occur. Since N cycling is controlled by a large set of parameters

360   such as pH, soil humidity, land-use, short and long-term variations in the predominant N

361   forms available for plants are to be expected. Under some conditions, plants can also take up

362   organic N (amino acids) directly and/or through fungi (Näsholm et al. 2000). While we

363   assumed that this source of N is of limited importance for our species in our relatively fertile

364   grasslands (Jones et al. 2005, Kahmen et al. 2009), a full understanding of the N preference,

365   and discussion about species coexistence through N forms sharing, would require careful

366   investigations, beyond the possibility in our study. Nonetheless, the variability we observed in

367   the ratio of uptake between the inorganic N forms suggested that, to some extent, plant

368   physiology was adjusted to match the soil conditions where species occurred. Yet, differences

369   between species with different strategies remains, with higher uptake for both forms

370   associated with a more exploitative strategy. We hypothesised that this should be also the case

371   for organic N sources (Kastovska and Santruckova 2011). Nevertheless, we acknowledge that

372   this question could be more important in harsh environments where organic N is relatively

373   more abundant as a N source for plants (Modzer et al 2014). Further investigations remain

374   needed on the variations of plant N uptake under field conditions, in link with potential

375   variations in N cycling in response to land-use or during the season (Robson et al. 2010,

376   Legay et al. 2013).

377

378   Variations of N uptake ability during the growing season

16

379   Plant N uptake ability also varies during the growing season, with N uptake increasing

380   (Stahl et al. 2011) or decreasing (Jaeger et al. 1999) depending on the ecosystems

381   investigated. In the UU grassland, NH_4^+ uptake was higher for all species during the autumn

382   than during the summer, and the same was found for NO_3^- uptake by *B. erectus*. Plant activity

383   is usually considered to slow down during the autumn compared to the peak biomass in

384   summer, an assumption supported by higher LDMC and lower SLA for all species related to

385   the senescence of leaves. However, we did not observe any changes for root traits, suggesting

386   that roots could remain active during this time of the growing season, especially in the process

387   of resource storage, an important feature for subalpine/alpine plants (Jaeger and Manson

388   1992, Kleijn et al. 2005). Additionally, studies have reported an increase of grasslands N

389   cycling rate in the autumn that could be explained by more favourable soil conditions (first

390   rains and mild temperature), and associated with still active N uptake by plants as observed in

391   our study (Grogan and Jonasson 2003, Miller et al. 2009, Larsen et al. 2012). Despite the fact

392   that only few soil parameters differed between seasons in the UU grassland, the N uptake

393   increase in autumn was more likely a site-dependent effect related to soil conditions (Miller et

394   al. 2009, Stahl et al. 2011, Legay et al. 2013) rather than a species response since they did

395   show the same pattern in the other grasslands.

396

397   Conclusions

398   By estimating inorganic root N uptake under controlled conditions from plants grown up

399   under field conditions, our results support the assumption that root and leaf functional traits

400   are associated with the ability of plants to acquire N. In particular, the observed pattern for

401   roots characteristics appeared similar to the one observed in the leaf economic spectrum, with

402   higher N uptake associated with more exploitative syndrome of traits. However, a weak

403   relationship between leaf and root traits suggests that leaf traits alone were insufficient to

17

404 predict N uptake. Additionally, N uptake varied within species during the growing season and

405 in response to local conditions, making root traits and soil parameters important features of

406 the relationships between plant functioning and grasslands N cycling.

407

412   References

413   Bahn M., Knapp M., Garajova Z., Pfahringer N., Cernusca A. (2006) Root respiration in
414   temperate mountain grasslands differing in land use. Global Change Biology **12**:995–
415   1006.

416   Bardgett R.D., Bowman W.D., Kaufmann R., Schmidt S.K. (2005) A temporal approach to
417   linking aboveground and belowground ecology. Trends in Ecology & Evolution
418   **20**:634–641.

419   Bardgett R.D., Streeter T.C., Bol R. (2003) Soil microbes compete effectively with plants
420   fororganic-nitrogen inputs to temperate grasslands. Ecology **84**:1277–1287.

421   Bardgett R.D., Streeter T.C., Cole L., Hartley I.R. (2002) Linkages between soil biota,
422   nitrogen availability, and plant nitrogen uptake in a mountain ecosystem in the
423   Scottish Highlands. Applied Soil Ecology **19**:121–134.

424   Bassirirad H. (2000) Kinetics of nutrient uptake by roots: responses to global change. New
425   Phytologist **147**:155–169.

426   Britto D.T., Kronzucker H.J. (2002) NH4+ toxicity in higher plants: a critical review. Journal
427   of Plant Physiology **159**:567–584.

428   Britto D.T., Kronzucker H.J. (2013) Ecological significance and complexity of N-source
429   preference in plants. Annals of Botany **112**:957–963.

430   Chapin F.S. (1980) The Mineral Nutrition of Wild Plants. Annual Review of Ecology and
431   Systematics **11**:233–260.

432   Cornelissen J.H.C., Lavorel S., Garnier E., Díaz S., Buchmann N., Gurvich D.E., Reich P.B.,
433   Steege H. ter, Morgan H.D., Heijden M.G.A. van der, Pausas J.G., Poorter H. (2003)
434   A handbook of protocols for standardised and easy measurement of plant functional
435   traits worldwide. Aust J Bot **51**:335–380.

436   Cornwell W.K., Cornelissen J.H.C., Amatangelo K., Dorrepaal E., Eviner V.T., Godoy O.,
437   Hobbie S.E., Hoorens B., Kurokawa H., Pérez-Harguindeguy N., Quested H.M.,
438   Santiago L.S., Wardle D.A., Wright I.J., Aerts R., Allison S.D., Van Bodegom P.,
439   Brovkin V., Chatain A., Callaghan T.V., Díaz S., Garnier E., Gurvich D.E., Kazakou
440   E., Klein J.A., Read J., Reich P.B., Soudzilovskaia N.A., Vaieretti M.V., Westoby M.
441   (2008) Plant species traits are the predominant control on litter decomposition rates
442   within biomes worldwide. Ecology Letters **11**:1065–1071.

443   Craine J.M., Froehle J., Tilman D.G., Wedin D.A., Chapin I.I.I. (2001) The relationships
444   among root and leaf traits of 76 grassland species and relative abundance along
445   fertility and disturbance gradients. Oikos **93**:274–285.

446   Craine J.M., Lee W.G. (2003) Covariation in leaf and root traits for native and non-native
447   grasses along an altitudinal gradient in New Zealand. Oecologia **134**:471–478.

448   Craine J.M., Lee W.G., Bond W.J., Williams R.J., Johnson L.C. (2005) Environmental
449   constraints on a global relationship among leaf and root traits of grasses. Ecology
450   **86**:12–19.

451 Dray S., Dufour A.-B. (2007) The ade4 Package: Implementing the Duality Diagram for
452   Ecologists. Journal of Statistical Software **22** [online] URL:
453   http://ideas.repec.org/a/jss/jstsof/22i04.html (accessed 13 January 2014).

454 Errebhi M., Wilcox G.E. (1990) Tomato growth and nutrient uptake pattern as influenced by
455   nitrogen form ratio. Journal of Plant Nutrition **13**:1031–1043.

456 Fort F., Jouany C., Cruz P. (2013) Root and leaf functional trait relations in Poaceae species:
457   implications of differing resource-acquisition strategies. Journal of Plant Ecology
458   **6**:211–219.

459 Freschet G.T., Cornelissen J.H.C., Van Logtestijn R.S.P., Aerts R. (2010) Evidence of the
460   "plant economics spectrum" in a subarctic flora. Journal of Ecology **98**:362–373.

461 Garnett T.P., Smethurst P.J. (1999) Ammonium and nitrate uptake by Eucalyptus nitens:
462   effects of pH and temperature. Plant and Soil **214**:133–140.

463 Gavito M.E., Curtis P.S., Mikkelsen T.N., Jakobsen I. (2001) Interactive effects of soil
464   temperature, atmospheric carbon dioxide and soil N on root development, biomass and
465   nutrient uptake of winter wheat during vegetative growth. Journal of Experimental
466   Botany **52**:1913–1923.

467 Grassein F., Lemauviel-Lavenant S., Lavorel S., Bahn M., Bardgett R.D., Desclos-Theveniau
468   M., Laîné P. Relationship between functional traits and inorganic nitrogen acquisition
469   among eight contrasting European grass species. Annals of Botany **In press**

470 Grigulis K., Lavorel S., Krainer U., Legay N., Baxendale C., Dumont M., Kastl E., Arnoldi
471   C., Bardgett R.D., Poly F., Pommier T., Schloter M., Tappeiner U., Bahn M., Clément
472   J.-C. (2013) Relative contributions of plant traits and soil microbial properties to
473   mountain grassland ecosystem services. Journal of Ecology **101**:47–57.

474 Jaeger C.H., Monson R.K. (1992) Adaptive significance of nitrogen storage in Bistorta
475   bistortoides, an alpine herb. Oecologia **92**:578–585.

476 Jaeger C.H., Monson R.K., Fisk M.C., Schmidt S.K. (1999) Seasonal partitioning of nitrogen
477   by plants and soil microorganisms in an alpine ecosystem. Ecology **80**:1883–1891.

478 Kahmen A., Livesley S.J., Arndt S.K. (2009) High potential, but low actual, glycine uptake of
479   dominant plant species in three Australian land-use types with intermediate N
480   availability. Plant and Soil **325**:109–121.

481 Kronzucker H.J., Siddiqi M.Y., Glass A.D.M., Kirk G.J.D. (1999) Nitrate-Ammonium
482   Synergism in Rice. A Subcellular Flux Analysis. Plant Physiology **119**:1041–1046.

483 Lainé P., Ourry A., Macduff J., Boucaud J., Salette J. (1993) Kinetic parameters of nitrate
484   uptake by different catch crop species: effects of low temperatures or previous nitrate
485   starvation. Physiologia Plantarum **88**:85–92.

486 Leffler A.J., James J.J., Monaco T.A. (2013) Temperature and functional traits influence
487   differences in nitrogen uptake capacity between native and invasive grasses.
488   Oecologia **171**:51–60.

489 Legay N., Grassein F., Robson T.M., Personeni E., Bataillé M.-P., Lavorel S., Clément J.-C.

(2013) Comparison of inorganic nitrogen uptake dynamics following snowmelt and at peak biomass in subalpine grasslands. Biogeosciences Discuss **10**:8887–8917.

Léon M., Lainé P., Ourry A., Boucaud J. (1995) Increased uptake of native soil nitrogen by roots of Lolium multiflorum Lam. after nitrogen fertilization is explained by a stimulation of the uptake process itself. Plant and Soil **173**:197–203.

Liu G., Freschet G.T., Pan X., Cornelissen J.H.C., Li Y., Dong M. (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. New Phytologist **188**:543–553.

Louahlia S., Lainé P., Thornton B., Ourry A., Boucaud J. (2000) The role of N-remobilisation and the uptake of NH4+ and NO3- by Lolium perenne L. in laminae growth following defoliation under field conditions. Plant and Soil **220**:175–187.

Lucash M.S., Eissenstat D.M., Joslin J.D., McFarlane K.J., Yanai R.D. (2007) Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities. Trees **21**:593–603.

Maire V., Gross N., Da Silveira Pontes L., Picon-Cochard C., Soussana J. (2009) Trade-off between root nitrogen acquisition and shoot nitrogen utilization across 13 co-occurring pasture grass species. Functional Ecology **23**:668–679.

McCormack L., Adams T.S., Smithwick E.A.H., Eissenstat D.M. (2012) Predicting fine root lifespan from plant functional traits in temperate trees. New Phytologist **195**:823–831.

McKane R.B., Johnson L.C., Shaver G.R., Nadelhoffer K.J., Rastetter E.B., Fry B., Giblin A.E., Kielland K., Kwiatkowski B.L., Laundre J.A., Murray G. (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature **415**:68–71.

Miller A., Bowman W. (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? Oecologia **130**:609–616.

Miller A.E., Schimel J.P., Sickman J.O., Skeen K., Meixner T., Melack J.M. (2009) Seasonal variation in nitrogen uptake and turnover in two high-elevation soils: mineralization responses are site-dependent. Biogeochemistry **93**:253–270.

Mommer L., Weemstra M. (2012) The role of roots in the resource economics spectrum. New Phytologist **195**:725–727.

Quétier F., Thébault A., Lavorel S. (2007) Plant traits in a state and transition framework as markers of ecosystem response to land-use change. Ecological Monographs **77**:33–52.

R Core Team (2012) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. [online] URL: http://www.R-project.org/

Reich P.B. (2014) The world-wide "fast–slow" plant economics spectrum: a traits manifesto. Journal of Ecology **102**:275–301.

Reich P.B., Walters M.B., Ellsworth D.S. (1997) From tropics to tundra: Global convergence

529   in plant functioning. Proceedings of the National Academy of Sciences **94**:13730 –
530   13734.

531   Robson T.M., Baptist F., Clément J., Lavorel S. (2010) Land use in subalpine grasslands
532   affects nitrogen cycling via changes in plant community and soil microbial uptake
533   dynamics. Journal of Ecology **98**:62–73.

534   Robson T.M., Lavorel S., Clement J.-C., Roux X.L. (2007) Neglect of mowing and manuring
535   leads to slower nitrogen cycling in subalpine grasslands. Soil Biology and
536   Biochemistry **39**:930–941.

537   Roumet C., Urcelay C., Díaz S. (2006) Suites of root traits differ between annual and
538   perennial species growing in the field. New Phytologist **170**:357–368.

539   Ryser P., Lambers H. (1995) Root and leaf attributes accounting for the performance of fast-
540   and slow-growing grasses at different nutrient supply. Plant and Soil **170**:251–265.

541   Stahl V.M., Beyschlag W., Werner C. (2011) Dynamic niche sharing in dry acidic grasslands
542   -a 15N-labeling experiment. Plant and Soil **344**:389–400.

543   Bar-Tal A., Aloni B., Karni L., Rosenberg R. (2001) Nitrogen Nutrition of Greenhouse
544   Pepper. II. Effects of Nitrogen Concentration and NO3: NH4 Ratio on Growth,
545   Transpiration, and Nutrient Uptake. HortScience **36**:1252–1259.

546   Tjoelker M.G., Craine J.M., Wedin D., Reich P.B., Tilman D. (2005) Linking leaf and root
547   trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493–
548   508.

549   De Vries F.T., Manning P., Tallowin J.R.B., Mortimer S.R., Pilgrim E.S., Harrison K.A.,
550   Hobbs P.J., Quirk H., Shipley B., Cornelissen J.H.C., Kattge J., Bardgett R.D. (2012)
551   Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial
552   communities. Ecology Letters **15**:1230–1239.

553   Warncke D.D., Barber S.A. (1973) Ammmonium and Nitrate Uptake by Corn (Zea mays L.)
554   as Influenced by Nitrogen Concentration and NH+4 /NO-3 Ratio1. Agronomy Journal
555   **65**:950.

556   Withington J.M., Reich P.B., Oleksyn J., Eissenstat D.M. (2006) Comparisons of structure
557   and life span in roots and leaves among temperate trees. Ecological Monographs
558   **76**:381–397.

559   Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavender-Bares
560   J., Chapin T., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K.,
561   Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas
562   M.-L., Niinemets U., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankov
563   V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E.J., Villar R. (2004) The
564   worldwide leaf economics spectrum. Nature **428**:821–827.

565   Zeller V., Bahn M., Aichner M., Tappeiner U. (2000) Impact of land-use change on nitrogen
566   mineralization in subalpine grasslands in the Southern Alps. Biology and Fertility of
567   Soils **31**:441–448.

568

569 **Table 1:** Description of the studied grasslands. Past and current land uses describe the former

570 and current management of these grasslands (see Quetier et al. 2006 for more information).

571 TMF: Terrace Mown and Fertilized, TU: Terrace Unmown not fertilized but lightly grazed,

572 TM: Unterraced Mown, UU: Unterraced Unmown but lightly grazed.

573

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

574

575

Table 2: Mean values ± 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 (mm².g^{-1})	LDMC (mg.g^{-1})	LNC (mg.g^{-1})	SRL (m.g^{-1})	RDMC (mg.g^{-1})	RNC (mg.g^{-1})
B. erectus	TU	Summer	**30.07 ± 0.8** [a]	277.6 ± 2.1 [c]	21.46 ± 0.2	249.86 ± 72.0	307.77 ± 22.4	6.06 ± 0.1
		Autumn	15.43 ± 1.1 [c]	**444.06 ± 16.8** [a]	12.09 ± 0.9	253.58 ± 34.3	303.5 ± 6.3	7.68 ± 0.4
	UU	Summer	**22.58 ± 0.7** [b]	305.06 ± 4.2 [c]	15.57 ± 0.8	328 ± 74.2	290.82 ± 21.7	7.17 ± 0.1
		Autumn	16.37 ± 1 [c]	**385.1 ± 11.5** [b]	19.28 ± 2.7	222.12 ± 75.4	288.32 ± 21.6	7.87 ± 0.5
D. glomerata	TMF	Summer	**34.06 ± 1.6** [a]	248.79 ± 17.7 [c]	**32.6 ± 3.4** [a]	426.22 ± 60.5	232.49 ± 20.5 [b]	7.28 ± 0.4
		Autumn	20.15 ± 2 [b]	**298.51 ± 2.5** [b]	19.41 ± 2.2 [b]	290 ± 48.6	249.09 ± 8 [b]	7.99 ± 0.3
	UU	Summer	26.17 ± 0.3 [ab]	265.78 ± 9.1 [bc]	**24.2 ± 1.4** [ab]	318.69 ± 63.8	254.08 ± 5.6 [b]	6.91 ± 0.5
		Autumn	24.34 ± 1.7 [b]	**389.69 ± 10.5** [a]	21.81 ± 2.5 [ab]	216.87 ± 44.7	**304.99 ± 8.8** [a]	7.16 ± 0.2
F. paniculata	UM	Summer	**23.26 ± 0.4** [a]	232.06 ± 0.6 [c]	**20.22 ± 1.9** [a]	195.85 ± 21.3 [ab]	300.1 ± 21.5	5.15 ± 0.4
		Autumn	8.78 ± 0.5 [b]	**433.87 ± 18.8** [a]	10.61 ± 1.4 [b]	153.37 ± 20.5 [b]	369.12 ± 21.7	4.32 ± 0.5
	UU	Summer	**21.36 ± 0.9** [a]	261.06 ± 15.6 [c]	14.52 ± 1.8 [ab]	166.21 ± 20.7 [ab]	380.91 ± 6.4	4.16 ± 0.1
		Autumn	11.76 ± 1.4 [b]	**369.71 ± 7.2** [b]	15.53 ± 3.6 [ab]	293.71 ± 54.5 [a]	366.84 ± 23.3	4.31 ± 0.1

Table 3. Pearson correlations between NH_4^+ and NO_3^- maximum uptake rates (Vmax), PCA axes, leaf and root traits. Significant values (p-value <0.05) are indicated in bold.

	VmaxNH$_4^+$	VmaxNO$_3^-$	axe1	axe2	ratio	SLA	LDMC	SRL	RDMC	LNC
VmaxNO$_3^-$	**0.93**									
axe1	**0.65**	**0.76**								
axe2	-0.28	-0.24	0.00							
ratio	-0.46	**-0.68**	**-0.64**	0.43						
SLA	0.37	0.53	**0.84**	0.38	-0.54					
LDMC	-0.17	-0.26	**-0.60**	**-0.74**	0.09	**-0.74**				
SRL	0.55	**0.70**	**0.81**	-0.14	-0.51	0.54	-0.34			
RDMC	**-0.68**	**-0.77**	**-0.91**	0.31	**0.75**	**-0.63**	0.37	**-0.71**		
LNC	**0.64**	**0.65**	**0.87**	0.10	-0.32	**0.69**	-0.51	**0.66**	**-0.74**	
RNC	**0.58**	**0.62**	**0.65**	**-0.70**	**-0.80**	0.34	0.08	0.50	**-0.83**	0.42

Table 4. Soil properties (mean values ± 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 Content (%)	Summer	**22.33 ± 0.71 [b]**	**18.33 ± 1.12 [c]**	**na**	**34.89 ± 1.20 [a]**
	Autumn	11.44 ± 0.33 [b]	7.25 ± 0.88 [c]	13.58 ± 1.18 [b]	17.74 ± 1.54 [a]
pH	Summer	8.01 ± 0.04 [a]	8.03 ± 0.04 [a]	n.a.	**6.31 ± 0.05 [b]**
	Autumn	7.98 ± 0.02 [a]	8.05 ± 0.04 [a]	5.85 ± 0.05 [b]	6.02 ± 0.08 [b]
Soil Organic Matter (%)	Summer	13.16 ± 0.48 [c]	14.39 ± 0.93 [b]	n.a.	18.42 ± 0.64 [a]
	Autumn	12.47 ± 0.65 [b]	11.38 ± 0.93 [b]	14.02 ± 0.43 [ab]	16.98 ± 1.29 [a]
C:N ratio	Summer	**14.91 ± 0.35 [a]**	14.02 ± 0.64 [a]	n.a.	12.10 ± 0.15 [b]
	Autumn	13.46 ± 0.39 [a]	13.43 ± 0.64 [ab]	11.63 ± 0.14 [b]	11.74 ± 0.43 [b]
TDN (µgN.g^{-1} soil)	Summer	20.83 ± 2.95 [b]	16.85 ± 1.33 [b]	n.a.	55.59 ± 12.15 [a]
	Autumn	**46.12 ± 4.44 [a]**	**46.13 ± 4.74 [a]**	34.54 ± 1.21 [ab]	46.65 ± 6.04 [a]
NO_3^- content (µgN.g-1 soil)	Summer	3.87 ± 0.48 [ab]	**5.63 ± 0.64 [a]**	n.a.	2.31 ± 0.53 [b]
	Autumn	2.99 ± 0.54 [a]	0.54 ± 0.10 [b]	n.d.	1.39 ± 0.66 [ab]
NH_4^+ content (µgN.g-1 soil)	Summer	11.19 ± 0.47 [a]	15.43 ± 1.82 [a]	n.a.	12.23 ± 1.77 [a]
	Autumn	10.07 ± 1.37 [a]	11.54 ± 1.55 [a]	6.97 ± 0.94 [a]	10.17 ± 1.92 [a]
NH_4^+:NO_3^- ratio	Summer	3.56 ± 0.65 [b]	2.97 ± 0.30 [b]	n.a.	7.85 ± 1.62 [a]
	Autumn	3.53 ± 0.36 [b]	**28.68 ± 10.7 [a]**	n.d.	12.9 ± 6.4 [ab]

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.
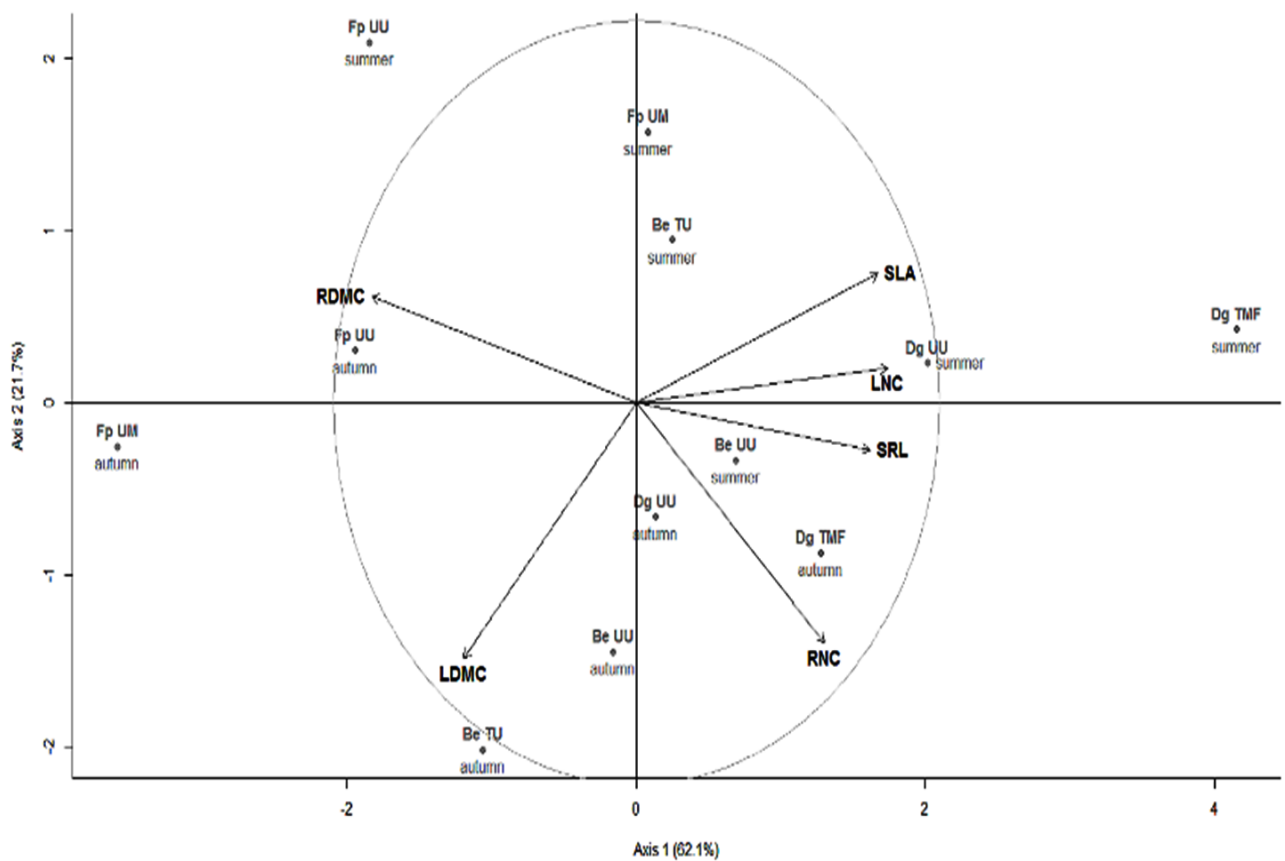
Figure 2: Relationships between the first axes of the PCA (fig 1) and Vmax for NH_4^+ (a), NO_3^- (b) and NH_4^+:NO_3^- uptake ratio (c). The three relationships were significant (p-values<0.05) assuming a polynomial relationship of order=2, and the resulting R^2 are indicated on each graph.
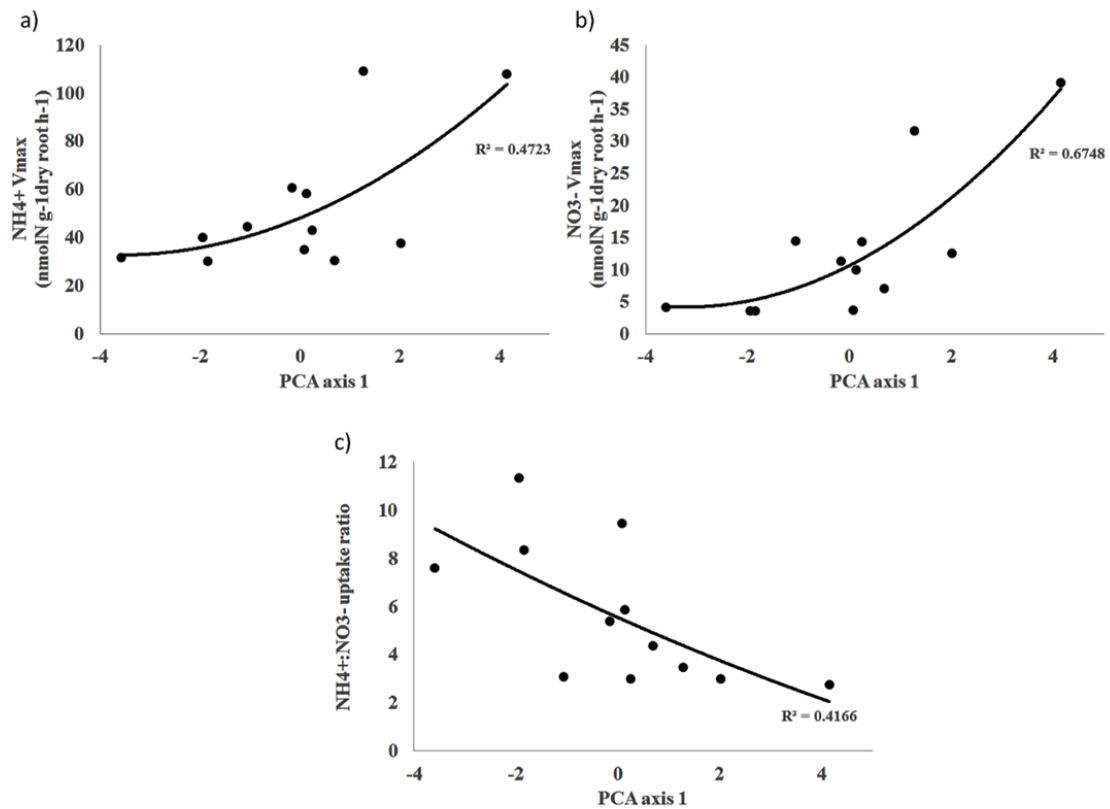
Figure 3. Vmax (Maximal uptake rate) for NH_4^+ (a) and NO_3^- (b) of *D. glomerata*, *B. erectus* and *F. paniculata*. Within each combination of site and species, dates with the same letter had similar uptake parameters (Tukey post hoc test at 5% level, after an Anova with date as main effect). For 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.
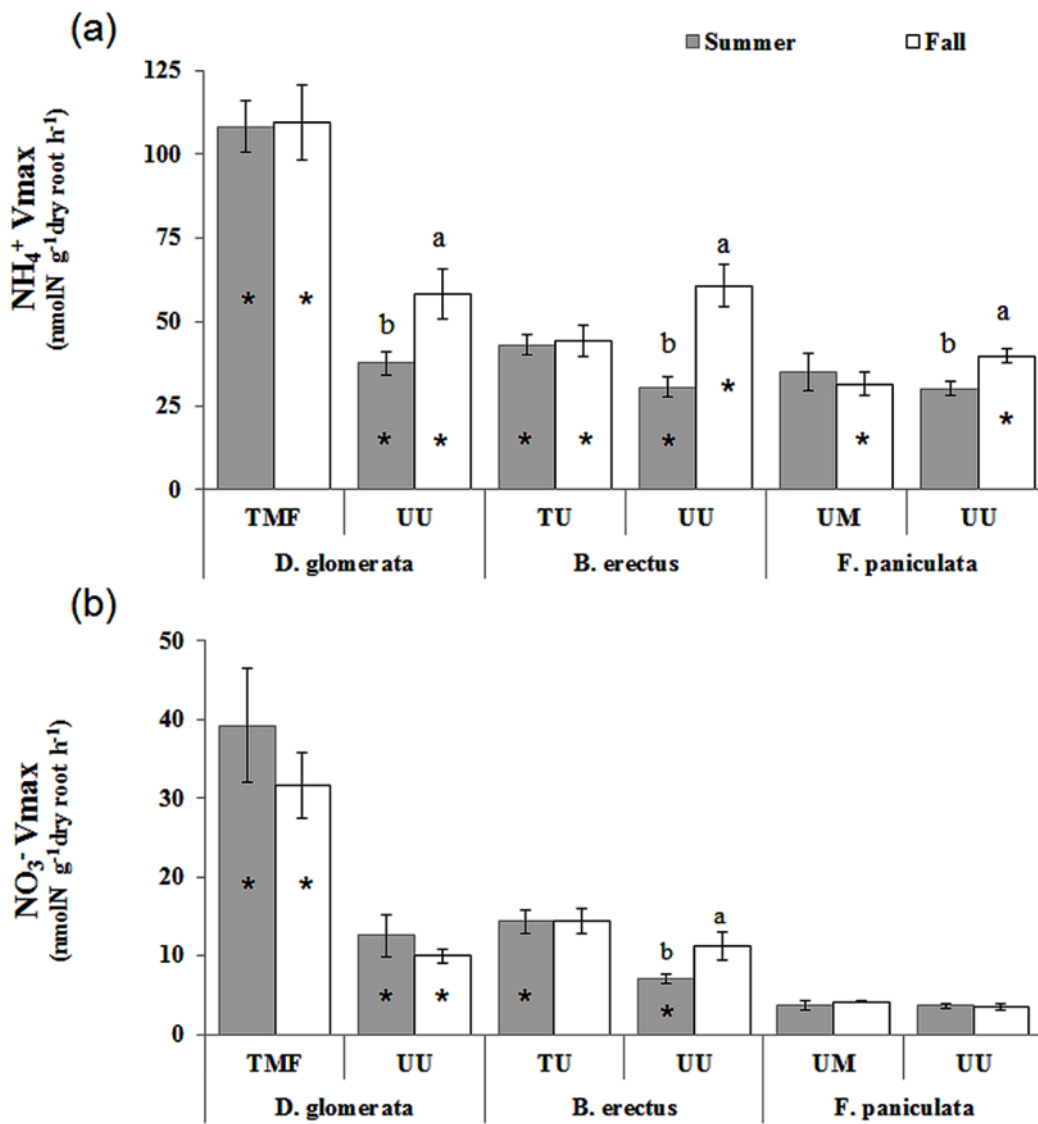
Figure 4: NH_4^+:NO_3^- uptake ratio for the three species, in each site and at the two sampling times. The uptake ratio is unitless (ratio between NH_4^+ Vmax and NO_3^- Vmax). Similar letters connect species with similar values across grasslands and dates. Pairwise tests were carried out using Tukey post-hoc tests at the error level of 5% following an ANOVA with date, species and site nested within species and the corresponding interactionswhich were not significant. Within each species, *or *** indicate significant site effects (p value < 0.05 and 0.001 respectively) within each species (ANOVA with site, date and the interaction as main effect).