



## Warming alters competition for organic and inorganic nitrogen between co-existing grassland plant species

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1 **Warming alters competition for organic and inorganic nitrogen between co-**  
2 **existing grassland plant species**

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27 **Abstract**

- 28
- Grass species may acquire different forms of nitrogen (N) to reduce competition for the same  
29 resources. Climate change influences the availability of soil N and is therefore likely to cause  
30 shifts in N forms acquired by plants, thereby affecting their competitive interactions.
  - We investigated the effects of warming on the uptake of different N forms and competitive  
31 interactions of *Festuca ovina* and *Anthoxanthum odoratum* in a pot experiment. The plants were  
32 grown either in monocultures or mixture, and at ambient or elevated temperature (+10 °C), and  
33 supplied with <sup>13</sup>C and <sup>15</sup>N isotopes to test for treatment effects on the relative uptake of  
34 ammonium, alanine or tri-alanine.
  - Both grass species took up relatively more N derived from ammonium than from alanine or tri-  
35 alanine when grown under ambient conditions in monoculture. In contrast, when grown in  
36 mixtures, *A. odoratum* took up N derived from the three N forms in equal amounts, whereas *F.*  
37 *ovina* switched to tri-alanine as an alternative N form. Under warmed conditions, both species  
38 took up the N forms equally, irrespective of competition treatments.
  - We have shown that grass species grown in mixture and under ambient conditions reduce  
39 competition by acquiring different N forms. Warming increased the availability of inorganic N  
40 in the soil and therefore deregulated the need for differential uptake of N forms.
- 41

44 **Keywords:**

45 amino acid, peptide, nutrient, coexistence, niche differentiation

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47 **Introduction**

48 Soil nitrogen (N) availability is one of the most important growth-limiting factors in natural or semi-  
49 natural grasslands (Vitousek and Howarth 1991). There is growing evidence that increasing  
50 temperatures due to global warming will accelerate rates of soil N turnover in these and other  
51 temperature-limited ecosystems (Bai et al. 2013; IPCC 2013; Prescott 2010; Zhang et al. 2008), leading  
52 to increased soil N availability and a shift in the dominant N form from dissolved organic N (DON) to  
53 soluble inorganic N (DIN) (Bai et al. 2013; Rennenberg et al. 2009; Saxe et al. 2001). In addition to  
54 changing climate, changes in grassland land use, such as shifts in management intensity or grazing  
55 density, also modify microbial communities and rates of soil N turnover, causing shifts in the availability  
56 of different N forms (de Vries et al. 2012; Medina-Roldan et al. 2012), with the amount of DON relative  
57 to DIN being greater in low than in high productivity, intensively managed grasslands (Bardgett et al.  
58 2003; Christou et al. 2005; Schimel and Bennett 2004).

59 It is well established that plant species are able to take up soil N in a range of forms, either as inorganic  
60 N, in the form of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), or as organic N, in the form of urea, amino acids  
61 and peptides (Näsholm et al. 2009; Näsholm and Persson 2001; Sauheitl et al. 2009b; Soper et al. 2011).  
62 Although grasses are relatively plastic with regard to their use of different N forms (Falkengren-Grerup  
63 et al. 2000; Sauheitl et al. 2009b), it has been suggested that under N limiting conditions grass species  
64 acquire contrasting forms of N, which appear to be linked to their growth strategies (Kahmen et al. 2006;  
65 Weigelt et al. 2005). This plasticity in acquiring different N forms has been proposed to be a strategy  
66 for co-existing plant species to reduce niche overlap, and therefore to avoid competition for the same  
67 limiting resource (Ashton et al. 2010; McKane et al. 2002). Results from studies testing for niche  
68 partitioning based on chemical forms of N in grasslands, however, are mixed: some report differences  
69 in N forms taken up by co-existing grassland plant species (Ashton et al. 2010; Kahmen et al. 2006),  
70 whereas others do not (Ashton et al. 2008; Harrison et al. 2007).

71 Given that climatic conditions are known to regulate the availability of different N forms, it is likely that  
72 modified N availability due to warming will also lead to a shift in N forms taken up by plants. Indeed,  
73 Warren (2009) reported that *Eucalyptus pauciflora* Sieber ex Spreng. took up more glycine than nitrate

74 at low temperatures, whereas the opposite was true when temperatures were higher due to changed N  
75 pool turnover rates. Similarly, in arctic tundra, glycine uptake by herbs was reduced by long-term  
76 warming (Sorensen et al. 2008), whereas glycine acquisition by the grass *Deschampsia flexuosa* (L.)  
77 was found to increase with warming (Andresen et al. 2009). Given this, our goal was to test how  
78 warming impacts the uptake of different N forms by grass species with contrasting life history strategies,  
79 and whether this influences their competitive interactions. We focused on two grass species that co-exist  
80 in low productivity, semi-natural temperate grassland: the slower-growing species *Festuca ovina* L. and  
81 the faster-growing species *Anthoxanthum odoratum* L. (Elberse and Berendse 1993; Ryser and Wahl  
82 2001; Schippers and Olf 2000; Schippers et al. 1999). These species have previously been shown to  
83 differ in their acquisition of organic and inorganic N forms in monoculture. *Festuca rubra* L., as a close  
84 relative to *F. ovina*, displays a selective placement in nutrient-rich patches with shorter roots and has  
85 been reported to take up relatively more inorganic than organic N, whereas *A. odoratum*, with its longer  
86 roots spread more evenly in the soil, relies equally on both forms (Elberse and Berendse 1993; Harrison  
87 et al. 2007; 2008; Mommer et al. 2011; Schippers and Olf 2000; Weigelt et al. 2005).

88 We hypothesised that: (i) when grown in monoculture, the two grass species would preferentially take  
89 up N derived from different N forms reflecting their differing life history strategies; (ii) when grown in  
90 mixture, this difference is amplified to avoid competition for soil N; and (iii) at warmer temperatures  
91 preferences for N derived from different forms becomes less important for *F. ovina* and *A. odoratum*  
92 due to increased availability of DIN compared to ambient temperatures. To test these hypotheses, we  
93 conducted a factorial pot experiment, in which *F. ovina* and *A. odoratum* were grown either in  
94 monocultures or mixtures at both ambient or elevated temperature, and were supplied with <sup>13</sup>C and <sup>15</sup>N  
95 labelled compounds to test the relative uptake of ammonium (as a representative form of inorganic N),  
96 alanine (amino acid) or tri-alanine (peptide).

97

## 98 **Materials and Methods**

### 99 *Experimental Setup*

100 We established a pot experiment using field soil collected from a grassland site at Abergwyngregyn,  
101 Gwynedd, North Wales, UK (53° 13' 27" N, 4° 00' 50" W, 320 m a.s.l.), as described by Farrell et al.  
102 (2011a). Briefly, the selected site is classified as a semi-natural *Agrostis-Festuca* grassland, based on  
103 the UK National Vegetation Classification (Rodwell 1992), and is dominated by the grasses *Agrostis*  
104 *canina* L., *Agrostis capillaris* L., *A. odoratum* and *F. ovina*, and the herbs *Potentilla erecta* (L.) Rausch.  
105 and *Galium saxatile* L.. The soil is an organic matter rich Cambic Podzol with an acidic pH (4.8) and is  
106 representative of a typical semi-natural, sheep-grazed upland grassland in the western United Kingdom  
107 (Bardgett et al. 2001). The dissolved N pool is rich in organic N ( $301 \pm 74 \text{ mg m}^{-2}$ ), whereas  
108 concentrations of  $\text{NH}_4^+$ -N ( $73.4 \pm 36.8 \text{ mg m}^{-2}$ ) and  $\text{NO}_3^-$ -N ( $0.6 \pm 0.5 \text{ mg m}^{-2}$ ) are lower (data refer to  
109 a depth of 15 cm, published in Wilkinson et al. (2015)). The climate, measured at sea level at a distance  
110 of ca. 1 km from the sampling site, is cool and wet with a mean annual air temperature of 10.7 °C, soil  
111 temperature of 11 °C (at 10 cm depth) and rainfall of 1250 mm. In spring 2013, soil from the field site  
112 was excavated from the rooting zone down to 15 cm depth. Soil was transported back to the laboratory  
113 where stones and roots were removed. After passing through a 4 mm sieve, the soil was thoroughly  
114 mixed and stored afterwards at 4 °C until the start of the experiment.

115 We selected two grass species: *A. odoratum* and *F. ovina*. Both species co-exist at the site, although *A.*  
116 *odoratum* is generally more abundant in more productive grasslands, and *F. ovina* is more abundant in  
117 lower productivity grasslands (Grime et al. 2007). In April 2013, seeds (Emorsgate Seeds, King's Lynn,  
118 UK) of *A. odoratum* and *F. ovina* were germinated in a 1:1 mixture (v:v) of a low fertility compost (No  
119 1; John Innes Manufacturers Association, Reading, UK) and horticulture sand (Keith Singleton  
120 Horticulture, Egremont, UK) at ambient temperatures in a greenhouse at The University of Manchester.  
121 Due to differences in germination and establishment rates, *A. odoratum* was sown two weeks later than  
122 *F. ovina* in order to produce uniformly sized seedlings. Trays were watered every second day with tap  
123 water without using any additional fertiliser. After 32 (*A. odoratum*) and 46 (*F. ovina*) days, seedlings  
124 with an average height of 9 cm were allocated to 3 intra- and interspecific planting treatments, each with  
125 two individual plants: i) *F. ovina* monoculture; ii) *A. odoratum* monoculture; and iii) *F. ovina* and *A.*  
126 *odoratum* mixture. Care was taken to ensure that the height of individuals in each of the 192 pots (side  
127 length = 9 cm, used height = 7 cm, average soil volume = 0.567 l) was similar. Immediately after

128 planting, pots of each treatment were randomly assigned to two temperatures in controlled growth  
129 cabinets (day length 16 h), namely: 12 °C, representing ambient growing season temperature, and 22  
130 °C, representing warming. The ambient temperature refers to an average temperature during growing  
131 seasons at the field site (13.7 °C at sea level, implying approximately 12 °C at the field site). Warming  
132 of 10 °C was used as an approach to extrapolate the climate sensitivity of N availability and uptake in a  
133 model ecosystem. Pots were randomly relocated within cabinets twice per week.

134 Pots were irrigated with tap water bi-weekly (ambient: 50 ml pot<sup>-1</sup> week<sup>-1</sup>; warming: 100 ml pot<sup>-1</sup> week<sup>-1</sup>;  
135 <sup>1</sup>, total dissolved N in tap water < 0.4 mg l<sup>-1</sup>), with differences in irrigation between the two treatments  
136 accounting for estimated greater evapotranspiration due to increased temperature and plant biomass in  
137 the warmed compared to ambient treatment. The difference in N input through irrigation between the  
138 treatments due to the different amount of water (ambient: < 0.16 mg pot<sup>-1</sup>; warming: < 0.32 mg pot<sup>-1</sup>)  
139 was negligible compared to total N per pot (approximately 2 g N pot<sup>-1</sup>). The height of each seedling  
140 (longest shoot) was measured weekly.

#### 141 *Isotope labelling and harvest of plant biomass*

142 Labelling of soils to measure uptake of different N forms was performed after 71 days, at a period when  
143 shoot height had remained stable for several weeks. Twelve replicate pots of each planting × temperature  
144 treatment were randomly allocated to the following three labelling treatments (72 out of 192 pots): i)  
145 <sup>15</sup>NH<sub>4</sub>Cl (98% <sup>15</sup>N, Cambridge Isotope Laboratories, Andover, MA, USA); ii) alanine (97-99% U-<sup>13</sup>C,  
146 97-99% <sup>15</sup>N, Cambridge Isotope Laboratories); and iii) tri-alanine, (97-99% U-<sup>13</sup>C, 97-99% <sup>15</sup>N, CK  
147 Gas Products, Ibstock, UK). Nitrate concentration in the original field soil was negligible compared to  
148 DON and ammonium (Wilkinson et al. 2015), and therefore, nitrate was not used for labelling. There  
149 were 4 replicates for each treatment-labelling combination. The other 120 pots were treated with an  
150 unlabelled N solution (18 μmol N pot<sup>-1</sup>), from which 8 pots were analysed for natural abundance  
151 assessments. Each labelling solution (18 μmol N pot<sup>-1</sup>) was made up of equal concentrations (6 μmol N  
152 pot<sup>-1</sup> for each N form) of ammonium, alanine and tri-alanine, in which one of the three N forms was  
153 isotopically labelled. This enabled us to test for preferential uptake by individual plant species and soil  
154 microbes (Harrison et al. 2007; Weigelt et al. 2005). The use of dual-labelled <sup>13</sup>C<sup>15</sup>N compounds is

155 generally, but not unequivocally, considered to be a good indication whether amino acids and peptides  
156 such as alanine and tri-alanine are taken up by plants directly as organic N, or as inorganic N after  
157 microbial mineralisation, as confirmed by enrichment of plant tissue with both  $^{13}\text{C}$  and  $^{15}\text{N}$  (Näsholm et  
158 al. 1998). The amount of N added to each pot was considered to be sufficient to allow for detection of  
159  $^{13}\text{C}$  and  $^{15}\text{N}$  within plant and microbial biomass, but keeping the possible N fertilisation effect on plant  
160 growth to a minimum ( $18 \mu\text{mol N pot}^{-1}$  ( $0.3 \text{ kg N ha}^{-1}$ )  $< \text{N}_{\text{H}_2\text{O}} = 490 \mu\text{mol N pot}^{-1}$ ). Within each pot, the  
161 labelling solution (20 ml) was injected at 5 different locations, equally distributed over the soil depth,  
162 using a glass syringe (S Murray & Co, Surrey, UK). Pots were randomly labelled over a period of four  
163 days.

164 Three hours after labelling, pots were destructively harvested and plants were separated from the soil.  
165 A chase period of 3 hours was chosen to reduce plant uptake of recycled mineralised organic N, but to  
166 provide sufficient time to detect  $^{13}\text{C}^{15}\text{N}$  in roots and shoots (Warren 2012). Roots were first washed with  
167 deionised water and then rinsed with 0.5 M  $\text{CaCl}_2$  to remove  $^{13}\text{C}$  and  $^{15}\text{N}$  in the apoplast and sorbed to  
168 the cell wall. Roots of the two species in the mixed treatment were distinguished from each other by  
169 their colour. Root, shoot and soil samples were dried at  $65^\circ\text{C}$  for two days prior to grinding (MM 400,  
170 Retsch, Haan, Germany). Root and shoot samples of the two individuals grown in monocultures were  
171 pooled prior to grinding, whereas for mixed treatments both individuals were analysed separately. In  
172 order to determine  $^{13}\text{C}^{15}\text{N}$  uptake by the soil microbial biomass, 0.5 M  $\text{K}_2\text{SO}_4$  extractions were carried  
173 out on fumigated (amylene-stabilised  $\text{CHCl}_3$ , Fisher Scientific, Waltham, MA, USA) and non-fumigated  
174 soil (Brookes et al. 1985). The extracts were freeze-dried prior to further processing (ScanVac CoolSafe  
175 55-4 Pro, Lyngø, Denmark). Microbial  $^{13}\text{C}^{15}\text{N}$  uptake was calculated as the respective differences  
176 between fumigated and non-fumigated samples. The differences were divided by the corrections factors  
177  $k_{\text{EN}} = 0.50$  and  $k_{\text{EC}} = 0.35$  to estimate microbial biomass C and N values (Carter 2008). However, we  
178 acknowledge the uncertainty in the values when used for isotopic labelling experiments (Glanville et  
179 al., 2016). For unknown reasons, we did not detect any uptake of  $^{13}\text{C}$  or  $^{15}\text{N}$  by the microbial biomass  
180 under warming, so data are not presented for this treatment.



181 Root, shoot, soil and microbial extract samples were analysed for  $^{12/13}\text{C}$  and  $^{14/15}\text{N}$  concentrations at the  
182 NERC Life Sciences Mass Spectrometer Facility, Centre for Ecology and Hydrology, Lancaster, UK,  
183 (precision for working standards better than 0.46 ‰ ( $^{13}\text{C}$ ) and 6.92 ‰ ( $^{15}\text{N}$ )). Samples were combusted  
184 in a Carlo Erba NA1500 elemental analyser (Thermo Scientific, Waltham, MA, USA). The resultant  
185  $\text{CO}_2/\text{N}_2$  from combustion and reduction was analysed for  $\delta^{13}\text{C}/^{15}\text{N}$  using an isotope ratio mass  
186 spectrometer (IRMS; Dennis Leigh Technologies, Sandbach, UK).  $^{13}\text{C}/^{15}\text{N}$  excess values were calculated  
187 by using formulas (1) and (2).

$$188 \quad R_{\text{sample}} = [(\delta^{13}\text{C}/1000) + 1] * R_{\text{PDB}} \quad (1)$$

189 where R is the ratio of  $^{13}\text{C}$ /of  $^{15}\text{N}$  to  $^{12}\text{C}$ /to  $^{14}\text{N}$  and  $R_{\text{PDB}}$  is the natural abundance standard for C and N.

$$190 \quad \text{Atom}\% = (R/R+1) * 100 \quad (2)$$

191 Atom % excess values were calculated by subtracting control atom % values from treatment atom %  
192 values. Natural abundance levels of  $^{13}\text{C}$  in our samples were highly variable. We therefore used the  
193 lowest natural abundance atom % value to calculate  $^{13}\text{C}$  excess values.

#### 194 *Soil nutrients, microbial biomass and root length*

195 Immediately after plants were harvested, fresh soil samples were extracted with deionised water (1:7.1  
196 w/v soil:extractant; extraction time = 10 min) to measure total dissolved N and inorganic N as either  
197 nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ). Extracts were measured with an AutoAnalyzer 3 (SEAL  
198 Analytical, Fareham, UK). DON was calculated after subtracting water-soluble inorganic N from total  
199 water-soluble N. Dissolved organic carbon (DOC) was measured in water extracts using a TOC-L  
200 analyser (Shimadzu, Kyoto, Japan). For determining microbial C ( $C_{\text{mic}}$ ) and N ( $N_{\text{mic}}$ ), chloroform-  
201 fumigated (fumigation time = 24 h) and non-fumigated soil samples were extracted with 0.5 M  $\text{K}_2\text{SO}_4$   
202 (1:2.5 w/v soil:extractant; extraction time = 60 min) (Brookes et al. 1985), and total soluble organic  
203 carbon and N in  $\text{K}_2\text{SO}_4$ -extracts were measured with a TOC-L (Shimadzu, Kyoto, Japan) and an  
204 AutoAnalyzer 3 (SEAL Analytical, Fareham, UK), respectively. In labelled soil samples, pH was  
205 measured in 0.01 M  $\text{CaCl}_2$  (FE20, Mettler-Toledo, Schwerzenbach, Switzerland). Root samples from  
206 pots that were not used for the labelling experiment were analysed for their diameter and length using

207 an Epson Expression 11000 XL, scanner (Nagano, Japan) and WinRHIZO Pro 2013a (Regent  
208 Instruments Inc., Quebec, CA).

#### 209 *Statistical analysis*

210 Data were analysed after log-transformation by ANOVA using a linear model (significant at  $P < 0.05$ )  
211 in R 3.02 (R Development Core Team, Vienna, AT). The initial shoot height (analysis of root and shoot  
212 biomass) and final biomass ( $^{12/13}\text{C}^{14/15}\text{N}$  values) were included in the models to account for differences  
213 between pots. Selected differences between treatments and soils were pair-wise tested using contrasts  
214 based on  $t$ -tests (significant at  $P < 0.05$ ).

## 215 **Results**

### 216 *Soil N availability and microbial biomass*

217 Concentrations of inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) were influenced by planting and warming treatments  
218 (Table 1). Concentrations of  $\text{NH}_4^+$  ( $F_{(2,181)} = 36.6$ ,  $P < 0.001$ ) and  $\text{NO}_3^-$  ( $F_{(2,181)} = 44.9$ ,  $P < 0.001$ ) were  
219 lowest in soil planted with *A. odoratum*, followed by mixtures and *F. ovina* monocultures. Soil  
220 concentrations of  $\text{NH}_4^+$  ( $F_{(1,181)} = 88.0$ ,  $P < 0.001$ ) and  $\text{NO}_3^-$  ( $F_{(1,181)} = 59.9$ ,  $P < 0.001$ ) were greater in  
221 the warming than in the ambient treatment. Dissolved organic N (DON) was lower in the ambient  
222 treatment than the warming treatment ( $F_{(1,181)} = 5.2$ ,  $P = 0.023$ ). Pair-wise comparisons for DON were,  
223 however, only significant in *A. odoratum* monocultures ( $P = 0.007$ ) and not in the other planting  
224 treatments (*F. ovina* monocultures:  $P = 0.171$ ; mixtures:  $P = 0.895$ ). Dissolved organic carbon (DOC)  
225 was changed by the planting treatment ( $F_{(2,186)} = 4.7$ ,  $P = 0.010$ ): DOC concentrations were, or tended  
226 to be greater in *A. odoratum* than in *F. ovina* monocultures (ambient:  $P = 0.054$ ; warming:  $P = 0.028$ ).  
227 There was, however, no warming effect on soil DOC, and neither total soil carbon ( $\text{C}_{\text{tot}}$ ), nitrogen ( $\text{N}_{\text{tot}}$ )  
228 or pH were affected by the warming and planting treatments (Table 1). Soil water concentration at the  
229 end of the experiment was greatest in warmed *F. ovina* monoculture, whereas no differences between  
230 the other treatments were observed.

231 Both microbial biomass C ( $F_{(1,167)} = 26.6, P < 0.001$ ) and N ( $F_{(1,167)} = 4.1, P = 0.045$ ) were greater in the  
232 ambient than in the warming treatment (Table 1). However, effects of warming on microbial biomass C  
233 (temperature  $\times$  planting:  $F_{(2,167)} = 11.8, P < 0.001$ ) and N (temperature  $\times$  planting:  $F_{(1,167)} = 5.5, P =$   
234  $0.005$ ) varied with planting design: under ambient conditions, microbial biomass C was lowest in  
235 mixtures, whereas under warmed conditions it was smallest in *F. ovina* monocultures. Similarly,  
236 microbial biomass N under warming was lower in *F. ovina* monocultures than in mixtures, but not  
237 different from *A. odoratum* monocultures. We observed no differences in microbial N between the  
238 planting treatments under ambient conditions.

#### 239 *Root and shoot biomass*

240 Elevated temperature on average doubled the shoot biomass of *A. odoratum*, whereas warming only  
241 marginally influenced shoot biomass of *F. ovina* (temperature  $\times$  species:  $F_{(1,247)} = 91.9, P < 0.001$ , Fig.  
242 1a). The planting treatment only affected shoot biomass of *A. odoratum* in the warming treatment  
243 (temperature  $\times$  planting:  $F_{(1,247)} = 4.5, P = 0.035$ ): *A. odoratum* shoot biomass per plant was 50% higher  
244 in mixtures than in monocultures (competition ratio (CR) =  $1.5 \pm 0.1$ ). However, total shoot biomass  
245 per pot (2 plants) in warmed *A. odoratum* monocultures did not differ from the total biomass of the two  
246 species in mixtures ( $P = 0.745$ ). Planting treatment had no effect on the shoot biomass of *A. odoratum*  
247 under ambient temperature (CR =  $0.9 \pm 0.1$ ), or on shoot biomass of *F. ovina* under ambient (CR =  $1.0$   
248  $\pm 0.1$ ) or warmed conditions (CR =  $1.2 \pm 0.1$ ).

249 Warming decreased root biomass of *F. ovina* in monoculture and mixtures, but it had no effect on root  
250 biomass of *A. odoratum* in monoculture, although it increased root biomass of this species in mixtures  
251 (temperature  $\times$  species:  $F_{(1,247)} = 49.7, P < 0.001$ , Fig. 1a). As a result, under elevated temperatures, root  
252 biomass of *A. odoratum* was more than four times greater than of *F. ovina*, whereas root biomass did  
253 not differ between the two species under ambient conditions (Fig. 1a). Warming decreased the root:shoot  
254 ratio of the test species ( $F_{(1,247)} = 178.9, P < 0.001$ ): the effect of temperature on the root:shoot ratio of  
255 *F. ovina* was greater than on that of *A. odoratum* (temperature  $\times$  species:  $F_{(1,247)} = 39.8, P < 0.001$ ),  
256 leading to a significantly higher root:shoot ratio of *A. odoratum* than of *F. ovina* in the warming  
257 treatment. There was no significant planting effect on root biomass or root:shoot ratio of either species

258 ( $F_{(1,247)} = 1.6, P = 0.201, F_{(1,247)} = 0.8, P = 0.383$ ). As with root biomass, root length of *F. ovina* was  
259 least in the warming than in the ambient treatment, whereas root length of *A. odoratum* grown in  
260 mixtures was greater under warming than ambient conditions (temperature  $\times$  planting:  $F_{(1,143)} = 55.4, P$   
261  $< 0.001$ , Fig. S1A). No warming effect on root length was observed in *A. odoratum* monocultures; hence,  
262 root length of *A. odoratum* was greater than of *F. ovina*, but only under warmed conditions.

#### 263 *N concentrations in root and shoot*

264 Temperature effects on shoot N differed between the two grass species (temperature  $\times$  species:  $F_{(1,87)} =$   
265  $37.1, P < 0.001$ ): shoot N in *A. odoratum* was greater under warming than under ambient temperature,  
266 whereas for *F. ovina* no effect of warming was detected (Fig. 1b). Planting design also influenced the  
267 two species differently (planting  $\times$  species:  $F_{(1,87)} = 19.4, P < 0.001$ ). Although pair-wise comparisons  
268 were not significant, shoot N in *A. odoratum* tended to be greater in mixtures than in monocultures,  
269 whereas it was the other way around in *F. ovina*. Hence, shoot N concentrations under ambient and  
270 monoculture conditions were higher in *F. ovina* than in *A. odoratum* ( $P = 0.004$ ), whereas N  
271 concentrations were lower in *F. ovina* than in *A. odoratum* in warmed mixture ( $P = 0.001$ ). In general,  
272 root N concentrations were greater under elevated than under ambient temperature ( $F_{(1,87)} = 9.1, P <$   
273  $0.001$ , Fig. 1b). However, pair-wise comparisons revealed that this response to warming was only  
274 significant in *A. odoratum* roots grown in mixtures ( $P = 0.015$ ).

#### 275 $^{13}\text{C}^{15}\text{N}$ excess values in root and shoot biomass

276 Enrichment of plant material, measured as absolute  $^{15}\text{N}$  excess values, differed strongly between the two  
277 grass species (roots:  $F_{(1,71)} = 21.1, P < 0.001$ ; shoots:  $F_{(1,71)} = 72.9, P < 0.001$ ). On average,  $^{15}\text{N}$  excess  
278 values in roots and shoots of *A. odoratum* were higher than in those of *F. ovina*, which is indicative of  
279 greater uptake of all N forms (Fig. 2, Table 2). Differences in  $^{13}\text{C}$  excess values between the two species,  
280 however, were only weakly or not significant (roots:  $F_{(1,47)} = 3.6, P = 0.064$ ; shoots:  $F_{(1,71)} = 5.8, P =$   
281  $0.020$ ), although there was a trend towards higher  $^{13}\text{C}$  concentrations in *A. odoratum* than *F. ovina* (Table  
282 2).

283 Plant uptake of N was affected by chemical N form (roots:  $F_{(2,71)} = 18.1, P < 0.001$ ; shoots:  $F_{(2,71)} = 22.2,$   
284  $P < 0.001$ ), planting design (roots:  $F_{(1,71)} = 10.0, P = 0.002$ ; shoots:  $F_{(1,71)} = 22.0, P < 0.001$ ) and warming  
285 treatment (roots:  $F_{(2,71)} = 6.5, P = 0.013$ ; shoots:  $F_{(2,71)} = 57.1, P < 0.001$ ). Most interestingly, planting  
286 treatment influenced the uptake of N forms by *A. odoratum* under ambient conditions: in monoculture,  
287  $^{15}\text{N}$  excess rates in *A. odoratum* roots and shoots were greater for ammonium than alanine (roots; shoots:  
288  $P < 0.001$ ;  $P < 0.001$ ) or tri-alanine ( $P = 0.066$ ;  $P = 0.023$ ), whereas in mixture uptake of N derived  
289 from tri-alanine was greater than from ammonium ( $P = 0.049$ ;  $P = 0.860$ ) or alanine ( $P = 0.011$ ;  $P =$   
290  $0.005$ ). This shift in N forms taken up by *A. odoratum* can mainly be deduced from a smaller ammonium  
291 uptake in mixture than in monoculture ( $P < 0.001$ ;  $P = 0.016$ ), whereas we observed no difference in  
292 uptake of N derived from tri-alanine between the planting treatments. In *F. ovina* roots and shoots grown  
293 at ambient conditions, differences between N forms were less obvious than for *A. odoratum*. In  
294 monoculture, uptake of N derived from alanine was less than for ammonium ( $P < 0.001$ ;  $P < 0.001$ ) and  
295 tri-alanine ( $P = 0.020$ ;  $P = 0.083$ ). In mixture, we observed no difference in uptake of different N forms  
296 on the basis of  $^{15}\text{N}$  excess in *F. ovina* roots, but values in shoots were greater when plants were labelled  
297 with ammonium than with alanine ( $P = 0.001$ ) or tri-alanine ( $P = 0.032$ ). Root uptake of  $^{13}\text{C}$  was also  
298 affected by the chemical form ( $F_{(1,47)} = 16.5, P < 0.001$ ); under ambient conditions  $^{13}\text{C}$  excess values  
299 were higher when plants were labelled with tri-alanine than with alanine (Table 2).

300 Warming changed the observed planting effects on  $^{15}\text{N}$  uptake under ambient conditions (temperature  $\times$   
301 form in roots:  $F_{(2,71)} = 3.0, P = 0.056$ ; shoots:  $F_{(2,71)} = 6.6, P = 0.002$ ); in general, we detected no  
302 differences in  $^{15}\text{N}$  and  $^{13}\text{C}$  excess values in both species between the applied N forms under warmed  
303 conditions (Table 2, Fig. 2). As an exception to this pattern,  $^{15}\text{N}$  excess values for *F. ovina* roots in  
304 monoculture were greater for tri-alanine than ammonium ( $P = 0.033$ ) or alanine ( $P = 0.023$ ), and for *A.*  
305 *odoratum* shoots,  $^{15}\text{N}$  excess values were greater for ammonium than alanine ( $P = 0.033$ ).

306 We found significant correlations between  $^{13}\text{C}$  and  $^{15}\text{N}$  excess values in *A. odoratum* roots for alanine  
307 ( $R^2 = 0.287, P = 0.027$ ) and tri-alanine ( $R^2 = 0.401, P = 0.011$ , Fig. 3). The slope of the alanine correlation  
308 line ( $m = 0.9$ ) was slightly steeper than that of tri-alanine ( $m = 0.5$ ), indicating that direct uptake of  
309 alanine was greater than for tri-alanine, that the proportion  $^{13}\text{C}$  lost in plant respiration was greater when

310 acquired as tri-alanine than when acquired as alanine, or that the C and N from the compounds  
311 partitioned differently between roots and shoots. In *F. ovina* roots, we observed no correlations between  
312  $^{13}\text{C}$  and  $^{15}\text{N}$  excess values (alanine:  $R^2 = 0.022$ ,  $P = 0.557$ ; tri-alanine:  $R^2 < 0.001$ ,  $P = 0.946$ ). The slopes  
313 of the correlation lines separately calculated for each planting and warming treatment did not differ from  
314 the patterns described above.

#### 315 $^{13}\text{C}$ $^{15}\text{N}$ excess values in microbes and soil

316 We observed significant differences under ambient conditions in microbial  $^{15}\text{N}$  excess values between  
317 the three N forms applied ( $F_{(2,54)} = 5.1$ ,  $P = 0.010$ );  $^{15}\text{N}$  excess values in microbes were greater when  
318 applied as tri-alanine than as ammonium (*F. ovina* monoculture:  $P = 0.011$ ; *A. odoratum* monoculture:  
319  $P = 0.054$ ; mixture:  $P = 0.056$ , Fig. 4). In *F. ovina* monoculture only, microbial  $^{15}\text{N}$  excess values  
320 originating from tri-alanine were also higher than those from alanine ( $P = 0.042$ ).  $^{15}\text{N}$  excess values in  
321 the case of ammonium solution application were close to zero, indicating that uptake of this N form by  
322 microbes was low. No data are presented for the warming treatment, as we did not detect any uptake of  
323  $^{13}\text{C}$  or  $^{15}\text{N}$  by the microbial biomass under warming,

324 In bulk soil samples, greater  $^{15}\text{N}$  excess values were observed when applied as tri-alanine than as  
325 ammonium or alanine ( $F_{(2,54)} = 42.9$ ,  $P < 0.001$ ). An exception to this pattern was that no difference in  
326 soil  $^{15}\text{N}$  excess values between the three labelling solutions was recorded for ambient *A. odoratum*  
327 monocultures (Fig. 4). Soil  $^{13}\text{C}$  data confirmed the pattern described above; when applied as tri-alanine,  
328 soil excess values were greater, or equal, than when applied as alanine ( $F_{(1,34)} = 29.8$ ,  $P < 0.001$ , Table  
329 2).

## 330 Discussion

331 The aim of this study was to test for the effects of warming on the uptake of different N forms and  
332 competitive interactions of two grass species of temperate grasslands with contrasting functional traits.  
333 Our first hypothesis was that the two grass species take up N derived from different N forms when  
334 grown in monocultures, and this difference is greater in mixture to avoid competition for soil N. In  
335 contrast to this hypothesis, and to previous studies on inorganic and organic N uptake (Harrison et al.

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336 2007; 2008; Weigelt et al. 2005), we found that both *F. ovina* and *A. odoratum* took up more N derived  
337 from ammonium from alanine when grown in monocultures. When grown in mixture, however, *A.*  
338 *odoratum*, but not *F. ovina*, switched from taking up more N from ammonium than alanine, to greater  
339 uptake of N derived from tri-alanine than from alanine or ammonium. The difference in N uptake  
340 between these species in mixture is reflected in their functional root traits: *Festuca* is known to place  
341 roots selectively in nutrient-rich hotspots, whereas *Anthoxanthum* spreads its roots more evenly in soil,  
342 allowing uptake of a greater variety of N forms (Mommer et al. 2011). This suggests that when grown  
343 in mixture, *F. ovina* was more competitive than *A. odoratum* in taking up the same N form as in  
344 monoculture, thereby reducing *A. odoratum*'s ammonium uptake. Competition for N between plants and  
345 microbes was presumably strong in both monocultures and mixtures, as indicated by higher <sup>15</sup>N excess  
346 values in microbes compared to plants, and, therefore, *A. odoratum* could not compensate the reduced  
347 ammonium uptake by acquiring more N derived from alanine or tri-alanine. Moreover, we exclude that  
348 competition between microbes and plants explains the decreased ammonium uptake by *A. odoratum* as  
349 this would likewise have affected ammonium uptake by *F. ovina*. Similarly, ammonium immobilisation  
350 by microbes did not differ between planting treatments under ambient conditions, as evidenced by the  
351 lack of change in microbial <sup>15</sup>N excess values derived from added ammonium. Our data, therefore,  
352 suggest that this shift in N uptake by *A. odoratum* was mainly induced by a lower competitiveness for  
353 ammonium in comparison with *F. ovina*, which lends support to the idea that acquisition of different N  
354 forms contributes to coexistence of competing grass species (Ashton et al. 2010; Kahmen et al. 2006;  
355 McKane et al. 2002).

356 As hypothesised, we found that warming changed N use by the two plant species, in that we detected no  
357 difference in uptake of the different N forms when they were grown in mixture compared to  
358 monocultures in this treatment. It is possible that increased soil inorganic N availability under warming  
359 compensated for the need for niche differentiation on the basis of N form, which was detected in  
360 mixtures under ambient conditions. Indeed, nitrate and ammonium concentrations were greater in the  
361 warming than ambient treatment, which is likely to be due to accelerated organic matter turnover in this  
362 organic-rich grassland soil (Bai et al. 2013; Prescott 2010; Rennenberg et al. 2009; Zhang et al. 2008).  
363 An alternative mechanism is that warming influenced the competitiveness of the two grass species,

364 which might have weakened in the requirement for niche differentiation; whereas under ambient  
365 conditions the biomass of the two species was similar, *A. odoratum* clearly outcompeted *F. ovina* in the  
366 warming treatment. In a study conducted by Schippers and Olf (2000), *A. odoratum* was still more  
367 vigorous than *F. ovina* at 15 °C, indicating that the optimum temperature of *F. ovina* is rather closer to  
368 12 °C than to 22 °C. The lower root:shoot ratio and plant N concentrations of *F. ovina* compared to *A.*  
369 *odoratum* indicate that the differences in competitiveness between our test species can be related to a  
370 more effective nutrient uptake by *A. odoratum* compared to *F. ovina* in the warmed treatment (Mommer  
371 et al. 2011). Otherwise, the low root biomass of *F. ovina* under warming might have been a consequence  
372 of the high soil water availability in the *F. ovina* monoculture relative to *A. odoratum* and mixtures. This  
373 would mean that due to sufficient water availability in the topsoil there was no need for *F. ovina* to  
374 allocate resources to root growth and hence *F. ovina* was likely less competitive in taking up nutrients  
375 compared to *A. odoratum*. With its higher root density, *A. odoratum* is likely to be also more competitive  
376 under water-limiting conditions, as predicted to increase in frequency with climate change (IPCC 2013);  
377 this question, however, was not tested in our experiment and needs further investigation. It is possible  
378 that, in the long term, niche partitioning on the basis of uptake of different forms of N will occur in the  
379 real world under warming, especially due to acclimatisation of microbial activity and increased plant  
380 biomass production (Lu et al. 2013; Luo et al. 2001) or immigration of other species (Klanderud and  
381 Birks 2003; Parolo and Rossi 2008). We therefore conclude, in accordance with our third hypothesis,  
382 that warming reduces the need for niche differentiation on the basis of N form in grass species, at least  
383 in the short timescale of our study.

384 Even though our data show how competition and temperature influence the uptake of N forms by *F.*  
385 *ovina* and *A. odoratum*, the applied <sup>13</sup>C<sup>15</sup>N labelling technique has some limitations. First, it is possible  
386 that N forms other than those we supplied to soil might have also been important for plant nutrition.  
387 Unlike in the field (Wilkinson et al. 2015), concentrations of nitrate were higher than ammonium or  
388 DON in soil of the present experiment. We found that soil nitrate concentrations were reduced under  
389 ambient conditions by the presence of *A. odoratum*, indicating that nitrate was a significant part of  
390 nutrition for *A. odoratum*. Soil concentrations of nitrate in mixtures, however, suggest that *A. odoratum*  
391 did not increase its nitrate acquisition when grown alongside with *F. ovina*. Hence, even though *A.*



392 *odoratum* may have taken up a significant amount of nitrate, our conclusions, gained from the reduced  
393 ammonium uptake in mixture compared to monoculture, would not be different. Second, correlations  
394 between  $^{13}\text{C}$  and  $^{15}\text{N}$  excess values in *A. odoratum* roots and  $^{13}\text{C}^{15}\text{N}$  excess values in microbes may  
395 indicate that a higher fraction of tri-alanine, compared to alanine, was first mineralised then taken up as  
396 inorganic N, as similarly reported by Farrell et al. (2013). We presume, however, that direct uptake of  
397 tri-alanine was nevertheless an important source for plant nutrition: on the one hand we found higher  
398 plant  $^{13}\text{C}$  excess values for tri-alanine than for alanine, indicating that direct uptake of the peptide was,  
399 in absolute numbers, higher than of the monomer; on the other hand, differences between  $^{13}\text{C}$  and  $^{15}\text{N}$   
400 correlations might be explained by faster within-plant mineralisation of tri-alanine compared to alanine  
401 (Hill et al. 2011; Warren 2012). In other words, residual carbon, including  $^{13}\text{C}$ , might have been respired  
402 to a higher extent when applied as peptide than as amino acid, resulting in a higher  $^{15}\text{N}^{13}\text{C}$  ratio. To  
403 reduce such uncertainties about direct uptake of labelled isotopes in future experiments, the application  
404 of other techniques might be helpful, such as compound-specific stable isotope measurements (Sauheitl  
405 et al. 2009a), position-specific labeling (Apostel et al. 2013) and the use of  $^{14}\text{C}$ -labelled isotopes (Hill  
406 et al. 2013). However, all available techniques are subject to some caveats and assumptions. Third, pool  
407 dilution of applied labelling solutions has to be taken into account when interpreting  $^{13}\text{C}$  and  $^{15}\text{N}$  uptake  
408 in plant samples (Jones et al. 2005). At field conditions, concentrations of alanine, tri-alanine and other  
409 amino acids and peptides competing for the same root transporters were smaller than those of  
410 ammonium in the soil used during the present pot experiment (Farrell et al. 2011a; Farrell et al. 2011b).  
411 Hence, the chance of a plant root to take up labelled ammonium was smaller in comparison with labelled  
412 N derived from organic forms. Otherwise, considering the faster turnover rates of amino acids and  
413 peptides comparing to ammonium, plants have much more capacity to take up  $^{15}\text{N}\text{-NH}_4^+$  during the  
414 labelling period. Taking pool dilution into account by multiplying soil N pools reported by Farrell et al.  
415 (2011b) by  $^{15}\text{N}$  excess values recorded within root or shoot tissue, we estimate that 30-100x more  $^{15}\text{N}\text{-}$   
416  $\text{NH}_4$  was recovered in plant material than the tested organic  $^{15}\text{N}$  forms. Likely, differences in N uptake  
417 in ambient monocultures would be even more distinct when considering pool dilution, whereas shifts in  
418 N uptake by *A. odoratum* grown in mixture would be less obvious. However, as this correction would

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419 likewise apply for both monocultures and mixture, the relative difference in ammonium uptake would  
420 not be different and hence, our main conclusions from this experiment are still likely to be true.

#### 421 *Conclusions*

422 Our data show that grass species grown in mixture and under ambient conditions reduce competition by  
423 taking up different N forms. Thereby, N derived from organic forms as amino acids and peptides can  
424 play a major role for plant nutrition. Hence, the possibilities for a plant species to create its own niche  
425 are manifold and may include intricacies such as acquiring different N forms. Increased availability of  
426 inorganic N due to warming deregulated the need for differential uptake of N forms. Hence, we conclude  
427 that uptake of different N forms is mainly important at nutrient-limiting conditions. Besides taking up  
428 different N forms, grass species have also been shown to coexist through spatiotemporal shifts in nutrient  
429 acquisition (McKane et al. 1990; Pomon et al. 2007). Whereas we exclude spatiotemporal shifts in N  
430 uptake as a source for niche differentiation in the present study, these other strategies might explain why  
431 in some field studies niche differentiation by taking up different N forms has been reported (Ashton et  
432 al. 2010; Kahmen et al. 2006), whereas in others it has not (Ashton et al. 2008; Harrison et al. 2007).

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580 **Figure legends**

581 **Fig. 1** a: Average root and shoot biomass per individual (g,  $\pm$  SE,  $n = 32$ ), separately for each temperature  
582 and planting treatment. Total root length is shown in Fig. S1. b: Average nitrogen (N) concentrations in  
583 root and shoot biomass (% ,  $\pm$  SE,  $n = 12$ ). Please note that the y-axis (shoot N) starts at 2%. a & b:  
584 Values of the two individuals in the monoculture treatment were pooled prior analysis. Significant ( $P <$   
585 0.05) pair-wise comparisons are indicated by \*: difference between monoculture and mixed treatments  
586 within the same species and temperature treatment; s: difference between species within the same  
587 temperature and competition treatment; w: difference between temperature treatments within the same  
588 species and competition treatment.

589 **Fig. 2** Average  $^{15}\text{N}$  excess rates in root (a) and shoot biomass (b) after a chasing period of 3 hours ( $\mu\text{mol}$   
590  $\text{g}^{-1}$ ,  $\pm$  SE,  $n = 4$ ), separately shown for  $\text{NH}_4^+$ , alanine and tri-alanine. Differences between the applied  
591 tracer solutions within a given treatment combination (column) are indicated by different lower/upper  
592 case letters (all  $P < 0.05$ ). Average  $^{13}\text{C}$  excess values are shown in Table 2. Please note the different  
593 scales between the two species.

594 **Fig. 3** Relationship between  $^{13}\text{C}$  and  $^{15}\text{N}$  excess values in roots of *A. odoratum* and *F. ovina* , separately  
595 shown for alanine (open circles) and tri-alanine (closed circles). Broken (alanine:  $R^2 = 0.287$ ,  $P = 0.027$ )  
596 and solid lines (tri-alanine:  $R^2 = 0.401$ ,  $P = 0.011$ ) show significant regressions between the excess of  
597 both isotopes in *A. odoratum* roots. The regressions in *F. ovina* roots were not significant (alanine:  $R^2 =$   
598 0.022,  $P = 0.557$ ; tri-alanine:  $R^2 < 0.001$ ,  $P = 0.946$ ). The dotted lines show the molar  $^{13}\text{C}:^{15}\text{N}$  ratios for  
599 the nitrogen sources injected (3:1).

600 **Fig. 4** Average  $^{15}\text{N}$  excess rates in bulk soil and microbes after a chasing period of 3 hours ( $\text{nmol g}^{-1}$ ,  $\pm$   
601 SE,  $n = 4$ ), separately shown for  $\text{NH}_4^+$ , alanine and tri-alanine. Differences between the applied tracer  
602 solutions within a given treatment combination are indicated by different letters (all  $P < 0.05$ ). No excess  
603 values are available for microbial samples in the warming treatment. Average  $^{13}\text{C}$  excess values are  
604 shown in Table 2.

605 **Supplementary Figures**

606 **Fig. S1** a: Average total root length per individual (m,  $\pm$  SE, ambient *F. ovina* & *A. odoratum*  
607 monocultures:  $n = 16$ , all other:  $n = 20$ ) and b: N content in roots and shoot (g,  $\pm$  SE,  $n = 12$ ), separately  
608 for each temperature and planting treatment. Significant ( $P < 0.05$ ) pair-wise comparisons are indicated  
609 by \*: difference between monoculture and mixed treatments within the same species and temperature  
610 treatment; s: difference between species within the same temperature and competition treatment; w:  
611 difference between temperature treatments within the same species and competition treatment.

## Tables

**Table 1** Soil properties at the end of the experiment. Values are mean  $\pm$  SE. Different letters indicate significant differences between competition treatments within the same warming treatment. SWC: soil water concentration (% ,  $n = 32$ , residual  $df = 183$ ),  $C_{tot}$ : total carbon ( $mg\ g^{-1}$ , 8, 42), DOC: dissolved organic C ( $\mu g\ g^{-1}$ , 32, 186),  $N_{tot}$ : total nitrogen ( $mg\ g^{-1}$ , 12, 66),  $NH_4^+$ : ammonium ( $\mu g\ g^{-1}$ , 32, 181),  $NO_3^-$ : nitrate ( $\mu g\ g^{-1}$ , 32, 181), DON: dissolved organic nitrogen ( $\mu g\ g^{-1}$ , 32, 181),  $Mic_C$ : microbial carbon ( $mg\ g^{-1}$ , 32, 167),  $Mic_N$ : microbial nitrogen ( $mg\ g^{-1}$ , 32, 167). An asterisk \* indicates a significant difference between warming treatments within the same competition treatment (all  $P < 0.05$ ). Statistical analyses ( $F$ -values): Effects of temperature (T,  $df = 1$ ), planting (P,  $df = 2$ ) and their interactions (T  $\times$  P,  $df = 2$ ), levels of significances (\*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ , \*:  $P < 0.05$ , (\*):  $P < 0.1$ )).

	Ambient			Warming			$F$ -values		
	<i>F. ovina</i> monoculture	<i>A. odoratum</i> monoculture	mixture	<i>F. ovina</i> monoculture	<i>A. odoratum</i> monoculture	mixture	T	P	T $\times$ P
SWC	* 41.1 $\pm$ 1.0 <sup>a</sup>	39.2 $\pm$ 1.2 <sup>a</sup>	38.0 $\pm$ 1.0 <sup>a</sup>	* 45.0 $\pm$ 1.6 <sup>a</sup>	31.2 $\pm$ 1.7 <sup>b</sup>	34.0 $\pm$ 2.1 <sup>b</sup>	9.6**	17.6***	8.3***
$C_{tot}$	90.1 $\pm$ 1.6 <sup>a</sup>	91.8 $\pm$ 0.7 <sup>a</sup>	92.5 $\pm$ 0.8 <sup>a</sup>	91.4 $\pm$ 0.6 <sup>a</sup>	91.2 $\pm$ 1.0 <sup>a</sup>	92.2 $\pm$ 1.9 <sup>a</sup>	<0.1	0.9	0.4
DOC	62.1 $\pm$ 5.7 <sup>a</sup>	73.3 $\pm$ 4.8 <sup>a</sup>	63.4 $\pm$ 5.1 <sup>a</sup>	56.4 $\pm$ 4.8 <sup>a</sup>	65.3 $\pm$ 2.9 <sup>b</sup>	59.2 $\pm$ 4.3 <sup>ab</sup>	1.5	4.7 *	0.1
$N_{tot}$	7.8 $\pm$ 0.1 <sup>a</sup>	7.8 $\pm$ 0.0 <sup>a</sup>	7.9 $\pm$ 0.0 <sup>a</sup>	7.9 $\pm$ 0.1 <sup>a</sup>	7.8 $\pm$ 0.1 <sup>a</sup>	7.9 $\pm$ 0.1 <sup>a</sup>	<0.1	0.8	0.5
$NH_4^+$	*2.7 $\pm$ 0.7 <sup>a</sup>	*1.3 $\pm$ 0.1 <sup>b</sup>	*1.6 $\pm$ 0.2 <sup>a</sup>	* 10.9 $\pm$ 1.7 <sup>a</sup>	*2.3 $\pm$ 0.3 <sup>b</sup>	*3.5 $\pm$ 0.5 <sup>b</sup>	88.0***	36.6***	8.7***
$NO_3^-$	*12.0 $\pm$ 2.0 <sup>a</sup>	*2.5 $\pm$ 0.5 <sup>b</sup>	*7.2 $\pm$ 1.6 <sup>a</sup>	* 57.9 $\pm$ 4.4 <sup>a</sup>	*6.5 $\pm$ 1.2 <sup>b</sup>	*14.9 $\pm$ 2.9 <sup>c</sup>	59.9***	44.9***	7.7***
DON	3.9 $\pm$ 0.3 <sup>a</sup>	*5.5 $\pm$ 1.0 <sup>a</sup>	4.0 $\pm$ 0.3 <sup>a</sup>	2.9 $\pm$ 0.6 <sup>a</sup>	*3.5 $\pm$ 0.3 <sup>a</sup>	4.1 $\pm$ 0.2 <sup>a</sup>	5.2*	2.2	2.1
$Mic_C$	*1.9 $\pm$ 0.1 <sup>a</sup>	*1.9 $\pm$ 0.1 <sup>a</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	*1.2 $\pm$ 0.1 <sup>a</sup>	*1.4 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.1 <sup>b</sup>	26.6***	2.1	11.8***
$Mic_N$	*0.29 $\pm$ 0.01 <sup>a</sup>	*0.30 $\pm$ 0.02 <sup>a</sup>	0.28 $\pm$ 0.01 <sup>a</sup>	*0.23 $\pm$ 0.01 <sup>a</sup>	*0.25 $\pm$ 0.01 <sup>ab</sup>	0.26 $\pm$ 0.01 <sup>b</sup>	18.5***	0.7	2.9(*)



**Table 2** Mean  $^{13}\text{C}$  excess values (nmol  $^{13}\text{C}$  excess  $\text{g}^{-1}$ ) in roots, shoots, soil and microbes, separately shown for the  $^{13}\text{C}$  labelling solutions alanine and tri-alanine. Values are mean  $\pm$  SE,  $n = 4$ . Different letters indicate significant differences between species and competition treatments within the same warming treatment and N form (all  $P < 0.05$ ). An asterisk \* indicates a significant difference between N forms within a given treatment. No excess values are available for microbial samples in the warming treatment.

	Ambient				Warming			
	<i>F. ovina</i> monoculture	<i>A. odoratum</i> monoculture	<i>F. ovina</i> mixture	<i>A. odoratum</i> mixture	<i>F. ovina</i> monoculture	<i>A. odoratum</i> monoculture	<i>F. ovina</i> mixture	<i>A. odoratum</i> mixture
<b>Roots</b>								
Alanine	279 $\pm$ 49 <sup>a</sup>	*235 $\pm$ 60 <sup>a</sup>	*129 $\pm$ 39 <sup>b</sup>	259 $\pm$ 50 <sup>a</sup>	439 $\pm$ 49 <sup>a</sup>	558 $\pm$ 113 <sup>a</sup>	348 $\pm$ 91 <sup>a</sup>	440 $\pm$ 109 <sup>a</sup>
Tri-alanine	474 $\pm$ 35 <sup>a</sup>	*475 $\pm$ 23 <sup>a</sup>	*268 $\pm$ 64 <sup>a</sup>	487 $\pm$ 67 <sup>a</sup>	567 $\pm$ 46 <sup>a</sup>	702 $\pm$ 83 <sup>a</sup>	397 $\pm$ 71 <sup>a</sup>	585 $\pm$ 103 <sup>a</sup>
<b>Shoots</b>								
Alanine	190 $\pm$ 82 <sup>a</sup>	269 $\pm$ 47 <sup>a</sup>	55 $\pm$ 111 <sup>a</sup>	310 $\pm$ 168 <sup>a</sup>	298 $\pm$ 41 <sup>a</sup>	428 $\pm$ 74 <sup>a</sup>	221 $\pm$ 149 <sup>a</sup>	324 $\pm$ 78 <sup>a</sup>
Tri-alanine	168 $\pm$ 50 <sup>a</sup>	292 $\pm$ 75 <sup>a</sup>	189 $\pm$ 134 <sup>a</sup>	212 $\pm$ 59 <sup>a</sup>	347 $\pm$ 46 <sup>a</sup>	475 $\pm$ 63 <sup>a</sup>	235 $\pm$ 58 <sup>a</sup>	347 $\pm$ 84 <sup>a</sup>
<b>Soil</b>								
Alanine	*17 $\pm$ 1 <sup>a</sup>	11 $\pm$ 3 <sup>b</sup>	*17 $\pm$ 3 <sup>a</sup>		*14 $\pm$ 1 <sup>a</sup>	*13 $\pm$ 2 <sup>a</sup>	18 $\pm$ 2 <sup>a</sup>	
Tri-alanine	*32 $\pm$ 2 <sup>a</sup>	13 $\pm$ 2 <sup>b</sup>	*27 $\pm$ 1 <sup>a</sup>		*26 $\pm$ 2 <sup>a</sup>	*22 $\pm$ 2 <sup>a</sup>	25 $\pm$ 6 <sup>a</sup>	
<b>Microbes</b>								
Alanine	11 $\pm$ 3 <sup>a</sup>	-4 $\pm$ 5 <sup>a</sup>	16 $\pm$ 9 <sup>a</sup>					
Tri-alanine	23 $\pm$ 14 <sup>a</sup>	17 $\pm$ 10 <sup>a</sup>	6 $\pm$ 1 <sup>a</sup>					