AEM Accepted Manuscript Posted Online 19 June 2015
Appl. Environ. Microbiol. doi:10.1128/AEM.01481-15
Copyright © 2015, American Society for Microbiology. All Rights Reserved.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

1 **Ectomycorrhizal communities on the roots of two beech (*Fagus sylvatica*) populations**
2 **from contrasting climate differ in nitrogen acquisition in a common environment**

3 Running title: Soil microbes and beech nitrogen uptake

4 Martin Leberecht[A], Michael Dannenmann[B,C], Silvia Gschwendtner[D], Silvija Bilela[C], Rudolf
5 Meier[B], Judy Simon[C], Heinz Rennenberg[C,E], Michael Schloter[D], Andrea Polle[A#]

6

7 *[A]Forest Botany and Tree Physiology, Büsgen-Institut, Büsgenweg 2, Georg-August-*
8 *University Göttingen, 37077 Göttingen, Germany*

9 *[B]Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-*
10 *IFU), Karlsruhe Institute of Technology (KIT), Kreuzeckbahnstrasse 19, 82467 Garmisch-*
11 *Partenkirchen, Germany*

12 *[C]Institute of Forest Science, Chair of Tree Physiology, University of Freiburg; Georges-*
13 *Koehler-Allee 53/54, 79110 Freiburg, Germany*

14 *[D] Research Unit Environmental Genomics, Helmholtz Zentrum München, German*
15 *Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764*
16 *Neuherberg, Germany*

17 *[E]King Saud University, PO Box 2454, Riyadh 11451, Saudi Arabia*

18

19 #Correspondence

20 Andrea Polle, email: apolle@gwdg.de, phone: +49(0)551 3933480, Fax: +49(0)551 3922705

21

22

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

23 **Abstract**

24 Beech (*Fagus sylvatica*), a dominant forest species in Central Europe, competes for nitrogen
25 with soil microbes and suffers from N limitation under dry conditions. We hypothesized that
26 ectomycorrhizal communities and the free living rhizosphere microbes from beech trees of
27 two contrasting climatic conditions exhibit differences in N acquisition that contribute to
28 differences in host N uptake and are related to differences in host below-ground carbon
29 allocation. To test these hypotheses young trees from the natural regeneration of two
30 genetically similar populations, one from dryer conditions (SW) and the other from cooler,
31 moist climate (NE) were transplanted into a homogeneous substrate in the same environment
32 and labelled with $^{13}CO_2$ and $^{15}NH_4^+$. Free living rhizosphere microbes were characterized by
33 marker genes for the N cycle, but no differences between the rhizosphere of SW or NE trees
34 were found. Lower ^{15}N enrichment was found in the ectomycorrhizal communities of NE
35 compared with the SW communities, whereas no significant differences were observed for
36 non-mycorrhizal root tips of SW and NE trees. Neither ectomycorrhizal communities nor non-
37 mycorhizal root tips showed differences in ^{13}C signatures between the NE and SW origins.
38 Because ^{15}N accumulation in fine roots and transfer to leaves were lower in NE compared to
39 SW trees, our data support that ectomycorrhizal community influence N transfer to their host
40 and demonstrate that the fungal community from the dry condition was more efficient in N
41 acquisition when environmental constraints were relieved. These findings highlight the
42 importance of adapted ectomycorrhizal communities for forest nutrition in a changing
43 climate.

44

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

45 **Introduction**

46 In Europe, forests are often confined to N-limited soils (1), where ectomycorrhizal fungi
47 (EMF) are key players for tree nutrition (2). Thus, not surprisingly many studies have
48 analyzed the composition of ectomycorrhizal communities and identified abiotic factors such
49 as drought as well as the quality and amount of host carbon allocation to roots as important
50 drivers for the structure of the ectomycorrhizal community (3–7). The control of EMF on N
51 flux is complex because on the one hand decreased host carbon supply results in decreased N
52 delivery by EMF, whereas high N availability leads to enhanced C transfer to the EMF (8–
53 10). On the other hand, EMF are instrumental to maintain host N supply under drought stress
54 and for the access to organic N from leaf litter (11, 12).
55 It has been realized that besides EMF the plant root interface including the rhizosphere is also
56 heavily colonized by bacteria and archaea. The same holds true for the mycorrhizal mantle
57 (13, 14). Besides other functions, these procaryotes are able to catalyze the mineralization of
58 organic matter. Other microbes like nitrifiers and denitrifiers compete with the plants for
59 nitrogen. Thus, bacteria and archaea also determine the nutrient supply of plants to a large
60 extent. Their community composition and activity pattern responds rapidly to changes in the
61 physiology of host plant both as a result of different plant developmental stages and shifts in
62 environmental conditions (15–18). For instance, microbial communities colonizing the root –
63 soil interface of beech changed their composition within few days after exposure of the plants
64 to elevated CO_2 probably as the result of an increased flow of plant derived assimilates into
65 the root and the surrounding soil (19). In another study where young field-grown beech trees
66 were transplanted from moist or dry environments to common edaphic and climatic
67 conditions, significant shifts in the abundance of transcripts coding for microbial genes
68 driving nitrification and denitrification were observed (19). Rapid changes in microbial
69 communities in response to shifts in the environmental conditions could be the reason why

Accepted Manuscript Posted Online

70 beech trees grown on similar soil type and N availability, but under different climatic
71 conditions exhibited differences in N uptake (20, 21). However, it is also obvious that due to
72 the highly complex network of interacting microbes in the rhizosphere predictions how
73 nutrient supply of the plants is influenced by differing environmental conditions are currently
74 not possible, mainly because individual members of these networks may respond differently
75 to changes and steer the complete network in unexpected directions.
76 The main goal of the present study was to elucidate whether functional differences for N
77 acquisition exist in ectomycorrhizal or rhizosphere microbial communities of trees from two
78 field sites with contrasting climatic conditions and whether these differences are related to
79 differences to N uptake of trees or microbes. In the present study we planted young, field-
80 grown beech trees, which were obtained from two contrasting sites with warm and dry or coo
81 and moist conditions, into the same soil. The plants were cultivated for two month under one
82 climatic scenario and then labelled with ^{15}N to determine N utilization and allocation between
83 soil, free microbes, EMF and plants. This treatment was chosen to improve our understanding
84 on the response pattern of the complete holobiont, i.e. the plant with its associated
85 microbiome, towards changes of the abiotic environment. As previous studies using the same
86 settings have shown that under common conditions, the community structures of bacteria at
87 the plant soil interface were comparable regardless the origin of the tree populations after two
88 month (19), we anticipated no differences in the microbial biomass and ^{15}N enrichment, if
89 there were no functional differences. In contrast to free microbes, differences in the species
90 composition of ectomycorrhizal communities were expected because the lifetime of
91 ectomycorrhizal roots lasts several months (22). We hypothesized that ectomycorrhizal
92 communities of trees originating from the dry forest are functionally divergent from those of
93 the moist forest and, therefore, differ in ^{15}N enrichment in a common environment. Because
94 preceding field studies showed reduced N uptake of trees at the dry site (21), we anticipated
95 reduced ^{15}N enrichment in the ectomycorrhizal communities of trees originating from the dry

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

96 forest compared with those from the moist forest even after two month of incubation under
97 comparable conditions. We have previously shown that ^{15}N enrichment in ectomycorrhizal
98 root tips is proportional to the flux of ^{15}N into the plant (12, 23). Therefore, we hypothesized
99 in addition that reduced ^{15}N enrichment in ectomycorrhizal communities results in reduced
100 ^{15}N enrichment in plant tissues and reduced whole-plant N uptake.

101 As the productivity of beech (*Fagus sylvatica* L.), a wide-spread, dominant forest species of
102 great economic value and ecological importance in Central Europe (24), is especially sensitive
103 to N limitation under environmental stress (25), we used this plant species for our
104 experiments. The path of N into different abiotic and biotic fractions was traced by using
105 $^{15}NH_4^+$. Because differences in photosynthesis and below-ground carbon allocation influence
106 the root-associated biota, soil respiration was measured and the distribution of novel
107 assimilated carbon in beech trees from the dry and moist forest was monitored after a 48h-
108 pulse of $^{13}CO_2$.

109

110 **Materials and Methods**

111 **Plant origin and plant cultivation**

112 Young beech (*Fagus sylvatica*) trees were collected from two 80 to 90-year-old beech-
113 dominated forests in the Swabian Jura near Tuttlingen (longitude 8°45'E, latitude 47°59'N,
114 South West Germany). The forests are located on the North East (NE) and the South West
115 (SW) exposure of a narrow valley (Krähenbachtal) with steep slopes (23°-30°) (26). The
116 genetic structures of young plants from the natural beech regeneration that were used for this
117 experiments, did not differ in NE from those in the SW forest (27). The mean long-term
118 annual regional air temperature (1961-1990) is 6.6 °C and the mean annual precipitation is
119 810 mm (20). Because of the higher irradiance on the SW site the soil temperature at 10 cm
120 depth is 0.8 °C higher and the soil water potential generally more negative than on the NE

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

121 site (28). The gravimetric soil water content, expressed as percentage of the maximum soil
122 water holding capacity was 54.1 ± 5.3% and 38.8 ± 3.4 % in the mineral soil of the NE and
123 SW sites, respectively. On both sites, the soil type is a shallow Rendzic Leptosol (sceletic)
124 (29) developed on limestone and marls of the jurassic Malm formation (30). Soil pH (water)
125 is 5.7 in the organic layer and 7.5 at 0.6 m depth (20). The concentration of NO_3^- ranged from
126 0.6 to 1.2 mg kg^{-1} dry soil and the concentration of NH_4^+ from 3.0 to 3.8 mg kg^{-1} dry soil.
127 Significant differences of the soluble N compounds between the soils of the NE and SW sites
128 were not observed. The organic carbon concentrations in the organic layer were similar with
129 46.0 ± 0.7 % at the SW and 43.5% ± 0.8 at NE site (26). The organic carbon concentrations
130 were higher in the mineral soil of SW than in that of NE (11.2 ± 0.8 % vs 8.5 ±0.6 %), with
131 both soils ranging at very high levels of SOC content (26).
132 Forty-five trees of stem heights of about 0.5 m were collected on each site. The collection
133 took place in July 2010 during the active phase of the season. The trees were excavated with
134 intact roots in their soil compartment and transported to a nursery (Forest Botanical Garden,
135 University of Göttingen, longitude 9°57'E, latitude 51°33'N). The root systems were carefully
136 cleaned under running water to remove adhering soil. The beech trees were planted separately
137 in 5 l containers (17 x 17 x 17 cm) in a homogenous mixture of coarse sand (0.7 – 1.2 mm
138 diameter, Melo Schwimmbadtechnik, Göttingen, Germany; 4.5 parts), fine sand (0.4-0.8 mm
139 diameter, Melo Schwimmbadtechnik; 4.5 parts) and peat (Torfwerk Zubrägel, Vechta,
140 Germany; 1 part). The sand was washed with tap water before use to remove small particles.
141 To match stand light conditions the trees were shaded with a 65 % shading net (Herrmann
142 Meyer KG, Rellingen, Germany) and placed outdoors. Each plant was watered daily with 50
143 ml tap water. Not all trees survived the transplantation, leaving 32 trees per site for the further
144 treatments. After one month, the beech trees were transferred into a greenhouse with 50 % air
145 humidity, an air temperature of 20 °C and long day conditions [16 h light achieved by
146 additional irradiation with MT 400 DL/BH lamps (Eye Iwasaki Electric Co. Ltd., Tokyo,

Accepted Manuscript Posted Online

147 Japan), resulting in a photosynthetically active radiation of 150 µmol m^{-2} s^{-1} quantum flux
148 density as determined by a photometer Li-185B equipped with a quantum sensor Li-190SB
149 (LiCor INC., Lincoln, USA). This light intensity is typical at tree height of young beech trees
150 in beech forests (31). Trees were cultured under these conditions for one month and watered
151 daily with 50 ml of a Hoagland-based nutrient solution (pH 5.7), which contained 0.4 mM
152 NH_4Cl as sole nitrogen source in addition to 0.05 mM $NaSO_4$, 0.1 mM K_2SO_4, 0.06 mM
153 $MgSO_4$, 0.13 mM $CaSO_4$, 0.03 mM KH_2PO_4, 0.005 mM $MnSO_4$, 0.005 $FeCl_3$, 5 µM H_3BO_3,
154 0.13 µM $NaMoO_4$, 0.18 µM $ZnSO_4$ and 0.16 µM $CuSO_4$ (adapted after (32)). Two days
155 before experimental labeling, root collar diameters at soil level, and heights of all trees were
156 measured.
157

158 **^{13}C and ^{15}N labeling**

159 The beech-soil systems were labeled with $^{13}CO_2$ and $^{15}NH_4Cl$. Continuous $^{13}CO_2$ (1.5 % ^{13}C,
160 Cambridge Isotope Laboratories, Andover, USA) fumigation started on the 4.10.12 at 8 am
161 and ended after 48 h. During this time the CO_2 concentration was continuously recorded and
162 exhibited a mean 1091 ± 193 ppm (UNOR 610 CO_2 analyzer, Maihak, Hamburg, Germany).
163 For homogenous distribution, the ^{13}C labeled air in the greenhouse was intensively mixed by a
164 fan (SF16R, SMC, Hong Kong, China).
165 $^{15}NH_4Cl$ (0.4 mmol, 99 % ^{15}N, Cambridge Isotope Laboratories, Andover, USA) was applied
166 daily on seven consecutive days starting on 4.10.12 at 8 am. To achieve homogenous ^{15}N soil
167 labeling, the nutrient solution was injected by syringes (1.5 ml) applying a total of 50.7 ml by
168 39 injections of 1.3 ml of ^{15}N-solution at depths of 0, 5 and 10 cm per container. This
169 treatment corresponds to a daily amount of 0.337 mg ^{15}N in excess of the natural abundance.
170 Eight trees per site were maintained in another greenhouse without ^{13}C and ^{15}N labeling as
171 non-labeled controls.
172

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online

173 **Soil respiration**

174 On day 2, 3, 4, 6 and 8 after the start of the experimental labeling, soil respiration and soil
175 $^{13}CO_2$ evolution were determined using a static chamber approach (33). At 11am, a chamber
176 (area: 120 cm^2, height 10 cm) was gently inserted 1 cm into the soil at 3 randomly chosen
177 containers of the NE and the SW treatment, respectively. Two minutes, 15 min and 30 min
178 after chamber placement, a gas sample of 20 ml was taken via gastight syringe sampling
179 through a septum. The air sample was immediately transferred in a septum-capped pre-
180 evacuated gastight 5 ml vial. For this purpose, the entire sample volume of 20 ml was flushed
181 from the syringe through the vial by using a second cannula in the septum cap, which was
182 removed at the end of the flushing procedure, leaving an overpressure of 25% in the sample
183 vial. Within four days after sampling, sampled air in vials was analyzed for the CO_2
184 concentration and the $\delta^{13}C$ signature of CO_2 using GasBench II coupled to the isotope ratio
185 mass spectrometer Delta Plus XP (Thermo Fisher Scientific, Bremen, Germany). We used a
186 PoraBOND Q column (Agilent, Böblingen, Germany) at 33 °C and a sample loop with 250
187 µL volume. Samples were calibrated using three standard gases (325 ppm CO_2 with $\delta^{13}C$ = -
188 8.296 ‰; 340 ppm CO_2 with $\delta^{13}C$= -29.3 ‰; and 550 ppm CO_2 with $\delta^{13}C$= -14.677 ‰ in
189 synthetic air) (Air Liquide, Kornwestheim, Germany). Soil respiration was calculated from
190 the increase in CO_2 concentrations during the 30 min sampling interval (33). Due to the low
191 soil respiration rates, the CO_2 concentration increase was linear over the 30 minutes period.
192 The increase in CO_2 concentration over time had to match a quality criterion of $R^2 > 0.9$;
193 otherwise the respective soil respiration flux was discarded (in one case).
194

195 **Harvest**

196 Eight plant-soil containers per site were harvested 1, 3 and 7 days after the start of ^{15}N
197 labeling. Eight non-labelled controls per site were harvested at d = 0. Leaves and above-
198 ground woody parts (branches and stem) were separated after harvest and weighed

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

199 immediately. To determine whole plant leaf area, an important parameter for photosynthesis,
200 five leaves were separately weighed, scanned with a CCD camera KP-C551 (Hitachi, Tokyo,
201 Japan) and analyzed with ImageJ 1.47v (National Institute of Health, Bethesda, USA). Whole
202 plant leaf area was calculated as: area of leaves/mass of leaves × mass of all leaves.
203 The roots were slightly shaken to remove loosely adhering soil. Rhizosphere soil, the soil
204 attached to roots after slight shaking, was sampled by a toothpick from the apical part of the
205 fine roots. Rhizosphere samples were weighed and immediately frozen on dry ice and stored
206 at -80°. The remaining root system was then carefully washed under running tap water.
207 Coarse (> 2 mm diameter) and fine roots (< 2 mm diameter) were separated and weighed.
208 One g of fine roots from unlabelled beech seedlings was shock-frozen in liquid N_2 for amino
209 acid determination. Fine root samples were wrapped in moist paper towels and stored in
210 darkness in plastic bags at 4°C. Dry mass of fine roots was determined after ectomycorrhizal
211 analysis. All remaining plant parts were weighed immediately after harvest, dried and
212 weighed after one week at 60°C.
213 The entire soil of each planting container was mixed and a representative sample of 500 g per
214 container was used for analyses in bulk soil.
215

216 **Soil analyses**

217 Immediately after harvest 300 g of representative bulk soil was weighed and dried at 105°C
218 for 24 hours. Soil water content (SWC [%]) was determined as:

219 $$SWC = \frac{sfw - sdw}{sdw} \times 100$$

220 with sdw being soil dry weight (g) and sfw soil fresh weight (g).
221 In order to quantify microbial biomass fresh soil aliquots of 60 g were either directly extracted
222 with 0.5 M K_2SO_4 solution at a soil:solution ratio of 1:2 or after 24 hours of fumigation with
223 chloroform (34). All extracts were filtrated with 0.45 µm syringe filters (Schleicher &
224 Schuell, Dassel, Germany) and immediately frozen. Aliquots of the soil extracts were used for

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

225 spectrophotometric determination of ammonium (NH_4^+) and nitrate (NO_3^-) concentrations by
226 a commercial laboratory (Dr. Janssen GmbH, Gillersheim, Germany) and for total dissolved N
227 (TN) and total dissolved organic C (DOC) by auto-analyzers as described by (34). Dissolved
228 organic N (DON) was calculated as the difference between TN in extracts and inorganic N in
229 extracts. Microbial biomass C and N was calculated from the difference in TN and DOC
230 between extracts from fumigated and unfumigated soil, without application of correction
231 factors (34).

232 In addition to N concentrations of the soil microbial biomass, NH_4^+, NO_3^- and DON pools,
233 their respective ^{15}N signatures were determined. The approach was based on diffusion of
234 NH_4^+ via pH increase on acid filter traps prepared for isotope ratio mass spectrometry, after
235 sequential conversion of all target-N compounds in soil extracts to NH_4^+ as described in detail
236 in earlier studies (35). Determination of ^{15}N enrichment in total extractable N of both
237 unfumigated control soils and fumigated soils was based on alkaline persulfate oxidation of
238 all N compounds to NO_3^- and subsequent NO_3^- reduction to NH_4^+ as described by (36).
239 Subsequent diffusion of NH_3 on acid filter traps enabled the quantification of ^{15}N enrichment
240 in microbial biomass as calculated from the difference in N concentration and ^{15}N enrichment
241 in TN between unfumigated control soils and fumigated soils (35). No correction factors were
242 applied in order to obtain estimates of ^{15}N uptake into active microbial biomass (37).

243

244 **Identification of ectomycorrhizal fungi**

245 The washed fine roots were spread under a dissecting microscope (205 FA, Leica, Wetzlar,
246 Germany) and remaining soil particles were removed using fine forceps. Randomly chosen 2
247 to 3 cm long root fragments were used for mycorrhizal analysis. Four hundred mycorrhizal
248 root tips per plant were counted and classified as mycorrhizal, non mycorrhizal or dead root
249 tips. If not enough root material was available to reach 400 mycorrhizal root tips, all available

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

250 root tips were counted. Aliquots of mycorrhizal, non-mycorrhizal and dead root tips were
251 collected of each plant and freeze-dried for ^{15}N and ^{13}C measurements.
252 The mycorrhizal root tips were assigned to morphotypes, based on morphological features
253 like color, mantle structure, type of ramification, shape of unramified ends, emanating hyphae
254 and rhizomorphes (38). All morphotypes were photographed with a DFC420 C camera (Leica,
255 Wetzlar, Germany) at 10 - 40 x magnification and aliquots were stored at -20°C for species
256 identification by internal transcribed spacer (ITS) sequencing.
257 The molecular identification of ectomycorrhizal fungi was conducted as previously described
258 in (5, 7)). We selected the abundant morphotypes to reach 90% coverage of the root tips. DNA
259 of about 20 pooled root tips assigned to one morphotype was extracted (innuPREP DNA Kit,
260 Analytik Jena, Jena, Germany) as recommended by the manufacturer. The ITS region was
261 amplified using the PCR primers ITS1F and ITS4 (Eurofins MWG Operon, Ebersberg,
262 Germany) (39, 40). The obtained PCR products were purified by precipitation (40µl deionized
263 water, 5 µl 5 M sodium acetate (pH 6.0), 150 µl 100 % ethanol) by centrifugation (25 min,
264 17,900 x *g*). The precipitate was washed once with 250 µl 100 % ethanol. Sequencing was
265 performed by the Sequencing service of the Büsgen-Institute, Department Forest Genetics and
266 Forest Tree Breeding of the Georg August University Göttingen using the Big Dye Terminator
267 3.1 Cycles Sequencing Kit (Applied Biosystems, Foster City, USA). The sequences were
268 assembled with StadenPackage V4.10 and compared with UNITE (http://unite.ut.ee) and
269 NCBI (http://www.ncbi.nlm.nih.gov) databases. Species names were accepted when 97 %
270 identities and a score over 800 bits were achieved. Details have been compiled in Table S1.
271 All sequences have been deposited in NCBI Genbank with GenBank accession numbers
272 KF498567-KF498582.
273
274 **C and N measurements and isotope analysis of plant tissues and mycorrhizal root tips**

Accepted Manuscript Posted Online

275 Dry plant tissues were ground with a ball mill (Retsch, Düsseldorf, Germany). Mycorrhizal,
276 non-mycorrhizal and dead root tips were processed without milling. Samples were weighed
277 (Supermicro S4; Sartorius, Göttingen, Germany) into 5 x 9 mm tin capsules (IVA
278 Analysetechnik, Meerbusch, Germany). For leaves 0.8 mg, for stem 5.0 mg, for coarse roots
279 3.0 mg, for fine roots 2.0 mg and for mycorrhizal, non-mycorrhizal and dead root tips 0.5-3
280 mg of tissue were used. ^{14}N, ^{15}N, ^{12}C, and ^{13}C isotope analyses were conducted at the service
281 unit KOSI (Kompetenzzentrum für Stabile Isotope, University Göttingen, Germany) on a
282 Delta Plus mass spectrometer (Finnigan MAT, Bremen, Germany; Interface: Conflo III,
283 Finnigan MAT, Bremen, Germany; elemental analyzer: NA2500, CE Instruments, Rodano,
284 Milano, Italy). APE (^{15}N atom-% excess) was determined as:

285 ^{15}N APE =atom-%$_{sample}$ – atom-%$_{natural\ abundance}$ with

$$\text{atom} - \% = \frac{^{15}\text{N}}{^{14}\text{N} + {}^{15}\text{N}} \times 100$$

286 Plant ^{15}N uptake (µg) was determined as 1000*(^{15}N APE$_{fine\ roots}$*10*N$_{fine\ roots}$ * dry mass of
287 fine whole-plant fine roots + ^{15}N APE$_{coarse\ roots}$*10*N$_{coarse\ roots}$ * dry mass of whole-plant coarse
288 roots) with N (mg g^{-1} dry mass$_{tissue}$). ^{15}N in leaves and stem+branches was neglected because
289 these tissues contributed only 0.4% and 0.7% to the whole plant uptake. Microbial ^{15}N uptake
290 (µg g^{-1} soil) was calculated as 1000*(^{15}N APE$_{(MBN)}$ *10*N)*soil mass in the container, with N
291 in mg N g^{-1} dry mass$_{soil}$ and 5520 g as the dry soil mass in the container.

292 δ^{13}C values were determined as:

293 $$\delta^{13}C = \left(\frac{\frac{^{13}C_{Sample}}{^{12}C_{Sample}}}{\frac{^{13}C_{VPDB}}{^{12}C_{VPDB}}}\right)$$ x 1000 with C$_{VPDB}$ = Vienna Pee Dee Belemnite Standard.

294 ^{13}C APE =atom-%$_{sample}$ – atom-%$_{natural\ abundance}$ with

$$\text{atom} - \% = \frac{^{13}\text{C}}{^{12}\text{C} + {}^{13}\text{C}} \times 100$$

295

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

296 **Quantification of microbes driving N - turnover**

297 DNA was extracted from 0.4 g of rhizosphere soil using the NucleoSpin Soil Kit (Macherey
298 Nagel, Düren, Germany) and the Precellys 24 Instrument (Bertin Technologies, Montigny-le-
299 Bretonneux, France). Quantity and quality of the extracted DNA were checked with a
300 Nanodrop spectrophotometer (PeqLab, Erlangen, Germany) and by gel electrophoresis (41).
301 The extracts were stored at -20°C until use.

302 Quantitative real-time PCR (RT-PCR) was performed using an ABI 7300 Cycler (Applied
303 Biosystems, Foster City, USA) to assess the abundance of selected marker genes which were
304 used as proxy for microbes involved in different steps of the nitrogen cycle with the following
305 assay reagents: DMSO and BSA (Sigma Aldrich), primers listed in Table S1 (Metabion,
306 Germany) and 2x Power SYBR Green master mix (Life Technologies, Darmstadt, Germany).
307 The respective 25 µl reaction mixtures for quantification of the genes [*nifH* (nitrogenase),
308 *amoA* AOA (ammonia monooxygenase in ammonia oxidizing archaea), *amoA* AOB (ammonia
309 monooxygenase in ammonia oxidizing bacteria), *narG* (nitrate reductase), *nirS* (nitrite
310 reductase), *nirK* (nitrite reductase), were composed as follows: 12.5 µl SYBR Green master
311 mix, 5 pmol of each primer, 0.5 µl 3% BSA and 2 µl DNA template. For the amplification of
312 *narG, nirK,* and *nirS* genes, 0.5 µl DMSO was added. Primer sources and measuring
313 conditions are summarized in Table S2.

314 For quantification, serial dilutions (10^1 to 10^6 gene copies μl^{-1}) of plasmid DNA containing
315 PCR products of the respective genes listed in Table S2 were used to calculate standard
316 curves. The PCR detection limit was assessed to 10 gene copies according to manufacturer's
317 instruction. To avoid PCR inhibition, the optimal dilution for each amplification assay was
318 determined in advance by dilution series of randomly chosen DNA extracts. The RT-PCR
319 assays were performed in 96-well plates (Life Technologies) for all target genes as described
320 in Table S2. All PCR runs started with a hot start at 95°C for 10 minutes. To confirm the
321 specificity of the SYBR Green-quantified amplicons, a melting curve analysis and a 1.5%

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

322 agarose gel were performed after each PCR run. The amplification efficiency was calculated
323 as Eff = $[10^{(-1/slope)}-1]$ and resulted in the following average efficiencies (standard deviation
324 less than 5% of mean): *nifH*, 87%, *amoA* AOA, 92%, *amoA* AOB, 86%, *narG*, 92%, *nirK*,
325 94%, *nirS*, 93%.
326

327 **Data analysis**

328 Data were analyzed with Origin Pro 8.5 (OriginLab Corporation, Northampton, USA) using
329 Students´ t tests for normal distributed data sets. Normal distribution was tested with the
330 Kolmogorov-Smirnov test. If data did not show normal distribution, they were log-
331 transformed to meet the requirement of normality. General Linear Models (GLM) were
332 applied to investigate the main factors (time, site) and their interactions (Statgraphics
333 Centurion XVI Version 16.2.03 (Statpoint Technologies, Warrenton, USA). In tables and
334 figures data are shown as means ± standard error (SE). Differences between means were
335 considered significant at $P \leq 0.05$. Species richness of ectomycorrhizal communities
336 (bootstrap of 200), ANOSIM (based on Morisita dissimilarity index), non-metric
337 multidimensional scaling (NMDS) and rarefaction curves were calculated with PAST 2.17c
338 (42).
339

340 **Results**
341

342 **Ectomycorrhizal communities at the plant-soil interface of beech trees originating from**
343 **two contrasting field sites after cultivation under common environmental conditions**

344 The root tips of young beech trees from NE and SW forests that were selected on the basis of
345 similar stem heights and diameters were about 30 to 38% colonized by EMF after two months
346 growth in a peat-sand-mixture in the same environment (Table 1). The ectomycorrhizal
347 fungal colonization of the NE roots was slightly higher than that of SW trees, while species

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

348 richness was higher on the SE roots (Table 1, rarefaction curve: Fig. S1). The fungal species
349 composition (shown in the supplement Table S1) was typical for the beech forest where the
350 young plants had been collected (6) (Fig. S2). The abundances of the ectomycorrhizal fungal
351 species differed between NE and SW trees (Table S1) resulting in significantly different
352 community structures (P of ANOSIM = 0.026). The ascomycete *Cenococcum geophilum* was
353 the most frequent fungal species on all trees and occurred twice as frequent on roots of
354 beeches from SW than on those from NE (Table S1). High abundances of *C. geophilum* were
355 also observed in previous field studies (6, 34).

356 About 55% of the root tips in our experiment were non-mycorrhizal and vital (Table 1),
357 whereas non-mycorrhizal roots were barely found on young trees directly after excavation
358 from their native soils (fraction of ectomycorrhizal root tips at NE and SW: 99.3 ± 0.4%, P_{site}
359 = 0.859).

360

361 **Carbon allocation pattern into the mycorrhizosphere of beech trees originating from**
362 **two contrasting field sites after cultivation under common environmental conditions**

363 The beech trees from the NE and SW forest were labeled in a greenhouse for 48h with $^{13}CO_2$.
364 During the labeling period neither microbial biomass nor the concentrations of organic and
365 inorganic soluble N compounds differed in the soil containing beeches from NE or SW (Table
366 S3).

367 To investigate carbon allocation of NE and SW trees, we determined the $\delta^{13}C$ signature in
368 leaves, roots and soil respiration as an integrative indicator for water availability and carbon
369 allocation (43). No significant differences of the $\delta^{13}C$ signatures were detected in beech leaves
370 from NE and SW before the start of the labeling (t = 0, Table 2). Therefore we have no
371 evidence for acute drought periods during their growth under field conditions prior to the
372 transfer to the greenhouse. ^{13}C pulse labeling did not indicate differences in photosynthetic
373 performance because the leaves from NE and SW beeches showed the same changes in the

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

374 $\delta^{13}C$ signature in leaves in response to the $^{13}CO_2$ labeling pulse at 8d (Table 2). However,
375 allocation of recent photosynthetate to fine roots was stronger in SW than in NE trees, evident
376 from a higher $\delta^{13}C$ signature in SW than in NE fine roots at 8d (Table 2).
377 The ^{13}C signatures of the ectomycorrhizas did not show differences between plants of SW or
378 NE origin (Table 2). The $\delta^{13}C$ signatures of the non-mycorrhizal root tips were much higher
379 than those of the ectomycorrhizas and any other tissue, but did not show an influence of plant
380 origin (Table 2). The signature of $\delta^{13}CO_2$ in soil respiration was also not significantly different
381 between NE and SE beech containers (Table S3), suggesting similar belowground utilization
382 of newly acquired photosynthetates. However, total soil respiration, which is the result of
383 microbial and root respiration, was higher in the soil from the NE than in the soil from SW
384 beeches (Table S3). The NE containers contained similar microbial biomass but higher root
385 biomass (Table S3). Furthermore, the fraction of dead root tips was lower on the NE than on
386 the SW plants and thus, root vitality of NE plants higher than that of the SW plants (Table 1).
387 Therefore, the increased respiration was most likely cause by the roots. Differences in root
388 vitality of the young trees with higher numbers of dead root tips at the SW than at the NE site
389 were also found on the roots of the mature trees at the field sites (fraction of dead root tips at
390 NE: 11 ± 2% and SW = 35 ± 7%, $P_{site} = 0.004$).
391

392 **Nitrogen dynamics in different compartments of beech trees originating from two**
393 **contrasting field sites after cultivation under common environmental conditions**

394 During the labeling period with $^{15}NH_4^+$, a significant time-dependent enrichment of ^{15}N was
395 found in all soil and plant fractions analyzed (P_{time} in the GLM for all data in Fig. 1 < 0.001).
396 Because the N concentrations of the soil, microbial and plant fractions did not change during
397 the time course of the experiment, the enrichment in ^{15}N also reflected ^{15}N accumulation in
398 those fractions. As expected, a strong ^{15}N enrichment appeared in the NH_4^+ soil solution (Fig.
399 1a), but NO_3^- and microbial biomass also showed strong ^{15}N enrichments indicating microbial

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

400 uptake and nitrification (Fig. 1b, d). In contrast, the ^{15}N enrichment in DON was about an
401 order of magnitude lower than in NO_3^- or soil microbes (Fig. 1c). Notably, the ^{15}N enrichment
402 was significantly higher in inorganic N compounds and in microbial N in the containers with
403 beeches from SW than in those with beeches from NE (Fig. 1a, b, d). The greatest difference
404 was found for NO_3^-, where the ^{15}N enrichment was about twice higher in containers with SW
405 trees than in those with NE trees after one week of labeling (Fig. 1b).
406 We also found stronger ^{15}N enrichments in fine roots and leaves of the SW than of the NE
407 beech trees (Fig. 1e, f). Overall, we recovered at day 8 of the ^{15}N treatment 262 ± 131 µg ^{15}N
408 and 337 ± 135 µg ^{15}N in NE and SW plants ($P = 0.06$) and 316 ± 111 µg ^{15}N and 451 ± 159
409 µg ^{15}N in the microbial biomass in NE and SW containers ($P = 0.09$), which correspond to
410 49% and 59% of the applied ^{15}N to the NE and SW plant-soil systems, respectively.
411 Because N acquired by the plant is taken up by the ectomycorrhizas and by the active zone of
412 the non-mycorrhizal root tips, we measured the ^{15}N enrichments in these tissues (Fig. 1g, h).
413 The ^{15}N enrichment in non-mycorrhizal root tips was very strong and exceeded that of ^{15}N in
414 the microbial fraction ($P= 0.004$), but without any significant differences between the NE and
415 SW trees (Fig. 1g).
416 In contrast to non-mycorrhizal roots tips, the ectomycorrhizas showed significant differences
417 in ^{15}N enrichment between SW and NE trees with stronger enrichment in SW than in NE trees
418 (Fig. 1h). The enrichment rate in the SW ectomycorrhizas was also stronger (slope: 0.63) than
419 in the microbes in the bulk soil of SW trees (slope: 0.31, $P_{slope} = 0.019$), whereas the
420 accumulation rates of ^{15}N in NE ectomycorrhizas and NE bulk soil microbes were slower
421 (slopes 0.24 for ectomycorrhizal and 0.29 for microbes) and not different ($P = 0.407$). The
422 difference in ^{15}N enrichment between the NE and SW ectomycorrhizal communities was
423 unlikely to result from differences in host carbon supply because the $\delta^{13}C$ signatures in the
424 NE and SW communities did not differ (Table 2).
425

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

426 **Abundance of microbes which drive the N turnover in the rhizosphere of beech trees**
427 **originating from two contrasting field sites after cultivation under common**
428 **environmental conditions**

429 To investigate whether microbial and plant N enrichment in the plant-soil system of NE and
430 SW originating trees were associated with functional differences in free living microbial
431 communities we determined the abundance of microbes driving nitrification, denitrification
432 and nitrogen fixation in rhizosphere soil based on marker genes (Fig. 2) As expected during
433 the pre-incubation period of the plants under common environmental conditions the
434 differences in the abundance pattern of the investigated microbial communities in N cycling,
435 which could be observed at the natural sites (SW and NE; data not shown) leveled out and no
436 significant differences could be observed between rhizosphere samples from trees of both
437 sites (Fig. 2). The only exception was the abundance of bacterial ammonia oxidizers, which
438 were significantly higher in rhizosphere samples from trees originating from the SW forest at
439 day 1 of the incubation experiment (Fig. 2b). However this effect disappeared at later time
440 points of the experiment. The application of NH_4^+ and the transient increase in atmospheric
441 CO_2 concentrations in the first 48h obviously triggered an increase of microbes involved in
442 denitrification, as the abundance of indicator genes for both nitrate and nitrite reducers (*narG*
443 respectively *nirK*) increased from day 0 (before application) to day1 and was then constant
444 over the complete experimental period (Fig. 2c,d). As a result of the increased levels of
445 nitrogen and carbon present in the rhizosphere bacterial ammonia oxidizers dominated over
446 archaeal ammonia oxidizers (*amoA* AOA) by one order of magnitude independent from the
447 sampling time point and the origin of the trees (data not shown). A similar observation was
448 made for nitrite reducers harboring the *nirK* respectively *nirS* genes. Here again, a dominance
449 of *nirK* harboring nitrite reducers was found in all samples (data not shown).
450
451 **Root performance, N uptake and ectomycorrhizal communities**

Accepted Manuscript Posted Online

452 To find out whether the differences in ectomycorrhizal communities between SW and NE
453 plants were related to N nutrition and plant performance such as N uptake, biomass and root
454 tip characteristics we conducted NMDS. The ordination illustrated the separation of the
455 ectomycorrhizal communities on SW and NE roots (Fig. 3). The correlation coefficients of the
456 variables are shown as vectors (Fig. 3). This analysis revealed that the separation of
457 ectomycorrhizal communities of SW and NE trees were related to plant ^{15}N uptake, microbial
458 ^{15}N uptake, the frequencies of dead root tips and ascomycota for the SW community and to
459 plant biomass and the fraction of ectomycorrhizal root tips for the NE fungal community (Fig.
460 3).
461

462 **Discussion**

463 **Tree origin does not impede N uptake**

464 Previous genetic and physiological studies with beeches from sites differing in water
465 availability revealed significant differences in tree performance suggesting that adaptation to
466 the environmental conditions may have occurred (44–47). Field studies, at the sites from
467 which the trees of the present study originated, showed that attached roots exposed to ^{15}N
468 containing soil solutions exhibited lower N uptake for the SW than for the NE beeches (21,
469 48). Because soil structure and soil N availability were similar in the NE and SW forest, it
470 was speculated that beech trees at drier sites might be physiologically or genetically impeded
471 for N uptake compared with beech populations from moist conditions (21). However,
472 population analysis with neutral genetic markers in the natural beech regeneration from the
473 dry SW and the moist NE sites did not show pronounced differentiation between the tree
474 origins (27). Furthermore, our results demonstrate no significant differences in whole-plant N
475 uptake when environmental constraints were relieved by culturing the NE and SW plants
476 under the same edaphic and climatic conditions. Therefore, our initial hypothesis that beech
477 trees from the dry forest are impaired in N uptake must be rejected.

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

478 In contrast to our expectation, beech trees from dry conditions showed even a higher N
479 enrichment in roots than those from the moist conditions. During the time course of our study
480 whole-plant N uptake was mainly confined to roots (98%). As the SW trees had significantly
481 less root biomass and more dead root tips than the NE trees, the flux of N into the SW trees
482 must have been higher than that into NE trees. This assumption is also supported by our
483 finding that the SW ectomycorrhizal community showed higher ^{15}N enrichment than the NE
484 assemblage. Therefore, the current results underpin that limited N uptake of the beech under
485 field conditions in the SW forest was caused biotic or abiotic environmental factors.

486 Plant carbon allocation to the root-rhizosphere system has been identified as an important
487 control for plant N provision (10, 34). However, higher carbon utilization as indicated by
488 higher respiration occurred in soil from NE than in that from SW trees. This might have been
489 due to the larger root system of NE trees or to higher turn-over of soil microbes. It is likely
490 that soil respiration in this experiment was dominated by the roots, because soil microbial
491 biomass was one to two orders of magnitude smaller than in forest soil under field conditions
492 (26, 30). The gross soil respiration was apparently fueled by stored carbon because the
493 signature of ^{13}C coming from new photosynthetate was unaffected. Overall, we have no
494 evidence that higher ^{15}N enrichment required higher instantaneous photosynthetic C
495 allocation to root tips, but the slightly higher ^{13}C signature in fine roots of SW trees suggests
496 that increased N nutrition may lead to C trade-offs in the long run.

497 Our results do not support that higher ^{15}N enrichment in the fine roots of SW compared with
498 NE trees was due to more efficient N uptake systems of the plants because no differences
499 were found for the non-mycorrhizal root tips of NE and SW trees. Internal ^{15}N reallocation
500 from ectomycorrhizal to non-mycorrhizal root tips is unlikely because previous studies
501 showed higher N uptake of non-mycorrhizal than of ectomycorrhizal beech trees (Pena et al.
502 2013b) and that ^{15}N enrichment in non-mycorrhizal root tips of mycorrhizal trees was similar

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

503 to that of non-mycorrhizal root tips of non-mycorrhizal trees (12). Assuming that the NO_3^-
504 and NH_4^+ levels per kg of dry soil found in the present study were completely dissolved in the
505 soil solution, the N concentrations were 290 to 360 µM for NH_4^+ and 39 to44 µM for NO_3^- in
506 the substrate of NE and SW trees. These NH_4^+ concentrations are in the optimal to excess
507 range for non-mycorrhizal beech roots (49) and in the saturation range of mycorrhizal roots of
508 field grown beeches (21), whereas NO_3^- is rather in the limiting range. Because of the
509 relatively high fraction of non-mycorrhizal root tips, which exhibited the highest ^{15}N
510 enrichment of all fractions regardless the plant origin, the overall influx into the roots might
511 have been higher than under field conditions, where the roots of the natural regeneration were
512 almost 100% mycorrhizal. However, the enhanced N uptake of non-mycorrhizal plants was
513 more vulnerable under stress than that of ectomycorrhizas (12).

514 It is further notable that non-mycorrhizal root tips exhibited the highest $\delta^{13}C$ signature of the
515 analyzed tissues, suggesting that they are strong sinks for energy, although we cannot exclude
516 that the extramatrical hyphae of the ectomycorrhizas were also strong C sinks. Preferential C
517 allocation to reward more cooperative symbionts of plants compared with less efficient ones
518 has been demonstrated for arbuscular mycorrhizas (50, 51). It is possible that this mechanism
519 also works for the non-mycorrhizal root tips, which were more ^{15}N-enriched than
520 ectomycorrhizas, but in ectomycorrhizas a relationship between ^{15}N and ^{13}C enrichment was
521 not found (23). In conclusion, our findings do not support that the differences in N enrichment
522 between the trees from the moist or the dry site were related to genetic differences of uptake
523 systems in non-mycorrhizal root tips or differences in below-ground carbon allocation of the
524 trees.

525

526 **Mycorrhizal and bacterial contributions to beech N supply**

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

527 The ^{15}N enrichment in the roots of the SW compared to the NE beeches was remarkable
528 because this finding suggests that N delivery by mycorrhizal and rhizosphere processes were
529 enhanced or that the competition by soil microbes was suppressed for the SW trees. In the
530 rhizosphere of SW and NE trees no evidence for differences in N cycling of the free living
531 microbes was found suggesting that pre-culture of the SW and NE trees in the same substrate
532 resulted in similar adjustments of microbial communities to the edaphic conditions. Under
533 field conditions, soil microbes are significant competitors for N (34, 35, 52) and acquire
534 soluble N faster than EMF (11). But here, the ^{15}N enrichment rates in microbes were similar
535 (NE) or even lower (SW) than those of the corresponding ectomycorrhizal communities, not
536 supporting competition under our conditions. Microbial completion was neither relevant
537 under N mass balance considerations due to an extremely low microbial activity in our soil
538 system compared to field conditions (here: 3 mg microbial N kg^{-1} dry soil *versus* 100 to 300
539 mg microbial N kg^{-1} dry soil in Tuttlingen forest soil (30, 34). The finding that about 20 % of
540 the added ^{15}N was recovered in soil microbial biomass and about 13% in the beech trees
541 further supports that microbial competition for N was relieved in our system because under
542 field conditions N uptake by microbial biomass is by more than one magnitude larger than by
543 young beech trees of the natural regeneration (35).

544 In contrast to free rhizosphere microbes, the common soil and climatic conditions did not
545 unify the ectomycorrhizal community composition. Ectomycorrhizal root tips have a lifespan
546 from months up to several years (53–55). Therefore, the observed dissimilarity of the fungal
547 communities still reflects differences of the SW and NE sites. The SW assemblage exhibited
548 higher ectomycorrhizal fungal species richness and stronger ^{15}N enrichment than the NE
549 assemblage. These superior features corresponded to higher ^{15}N enrichment in the fine roots
550 of SW trees and higher transport to the leaves and therefore support that the ectomycorrhizal
551 communities influence N delivery to the host. Apparently, the structures of divergent

552 ectomycorrhizal communities can play decisive roles in the observed variations in beech N
553 uptake and this may have resulted in differences in N uptake under field conditions (56). The
554 finding that divergent *in situ* ectomycorrhizal communities exhibit functional diversity is an
555 important result, because variation in ectomycorrhizal fungal species composition and
556 abundance has often been interpreted to reflect the adaptation of the fungi to fluctuations of
557 the external conditions, but the consequences for the host were unknown. The correlation of
558 plant ^{15}N uptake with the ectomycorrhizal community of SW trees and not with the higher
559 plant biomass of the NE trees supports that the SW fungal assemblage was more active in N
560 acquisition.

561 In conclusion, among the different components of the experimental soil-mycorrhizosphere-
562 plant system, the composition of the ectomycorrhizal community was related to host N
563 acquisition, whereas evidence for physiological differences as the result genetic adaptation or
564 long lasting acclimation due to plant origin was not observed. Our findings highlight that the
565 associated ectomycorrhizal communities of beech from the two contrasting environments
566 exhibit important differences in N acquisition in the soil-plant system. How different
567 ectomycorrhizal communities interact with the complex rhizosphere net of microbes under
568 field conditions and how a wider range of climatic effects impacts the functional composition
569 of ectomycorrhizal communities needs to be studied in future. To counteract the predicted
570 negative consequences of climate change for forest productivity (57), the identification of
571 drought tolerant beech provenances for the establishment of resistant forests has been
572 suggested (47, 58). Our results suggest that this strategy will be insufficient when not
573 combined with the application of adapted microbial communities, in particular
574 ectomycorrhizal communities, because these communities impact on tree N supply, and
575 therefore are crucial for forest productivity in N limited ecosystems.

576

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

577 **Acknowledgements**

578 We are grateful to the German Research Foundation/Deutsche Forschungsgemeinschaft
579 (DFG) for funding this work under contract numbers Po 362/19-1, RE 515/33-1, Da 1217/2-1,
580 SCHL446/12-1. Judy Simon was financially supported by the European Social Fund and by
581 the Ministry of Science, Research and the Arts (Baden-Württemberg).We would also like to
582 thank Dr. Jens Dyckmans (Kompetenzzentrum für Stabile Isotope, University of Göttingen)
583 for isotope analyses, Thomas Klein for ITS sequencing and Maximilian Huber for support
584 during the experimental work.

585

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

586 **Literature**

587 1. **Solberg S, Dobbertin M, Reinds GJ, Lange H, Andreassen K, Fernandez PG, Hildingsson A, de**
588 **Vries W**. 2009. Analyses of the impact of changes in atmospheric deposition and climate on
589 forest growth in European monitoring plots: A stand growth approach. For Ecol Manag
590 **258**:1735–1750.

591 2. **Read DJ, Perez-Moreno J**. 2003. Mycorrhizas and nutrient cycling in ecosystems–a journey
592 towards relevance? New Phytol **157**:475–492.

593 3. **Shi L, Guttenberger M, Kottke I, Hampp R**. 2002. The effect of drought on mycorrhizas of beech
594 (Fagus sylvatica L.): changes in community structure, and the content of carbohydrates and
595 nitrogen storage bodies of the fungi. Mycorrhiza **12**:303–311.

596 4. **Buée M, Vairelles D, Garbaye J**. 2005. Year-round monitoring of diversity and potential
597 metabolic activity of the ectomycorrhizal community in a beech (Fagus silvatica) forest
598 subjected to two thinning regimes. Mycorrhiza **15**:235–245.

599 5. **Druebert C, Lang C, Valtanen K, Polle A**. 2009. Beech carbon productivity as driver of
600 ectomycorrhizal abundance and diversity. Plant Cell Environ **32**:992–1003.

601 6. **Pena R, Offermann C, Simon J, Naumann PS, Geßler A, Holst J, Dannenmann M, Mayer H,**
602 **Kogel-Knabner I, Rennenberg H, Polle A**. 2010. Girdling Affects Ectomycorrhizal Fungal (EMF)
603 Diversity and Reveals Functional Differences in EMF Community Composition in a Beech Forest.
604 Appl Environ Microbiol **76**:1831–1841.

605 7. **Lang C, Seven J, Polle A**. 2011. Host preferences and differential contributions of deciduous tree
606 species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza
607 **21**:297–308.

608 8. **Corrêa A, Strasser RJ, Martins-Loução MA**. 2008. Response of plants to ectomycorrhizae in N-
609 limited conditions: which factors determine its variation? Mycorrhiza **18**:413–427.

610 9. **Albarracín MV, Six J, Houlton BZ, Bledsoe CS**. 2013. A nitrogen fertilization field study of
611 carbon-13 and nitrogen-15 transfers in ectomycorrhizas of Pinus sabiniana. Oecologia
612 **173**:1439–1450.

613 10. **Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg**
614 **MN**. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree
615 growth in boreal forests? New Phytol **198**:214–221.

616 11. **Pena R, Tejedor J, Zeller B, Dannenmann M, Polle A**. 2013. Interspecific temporal and spatial
617 differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages.
618 New Phytol **199**:520–528.

619 12. **Pena R, Polle A**. 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages
620 for nitrogen acquisition under stress. ISME J.

621 13. **Mogge B, Loferer C, Agerer R, Hutzler P, Hartmann A**. 2000. Bacterial community structure and
622 colonization patterns of Fagus sylvatica L. ectomycorrhizospheres as determined by
623 fluorescence in situ hybridization and confocal laser scanning microscopy. Mycorrhiza **9**:271–
624 278.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

625 14. **Schloter M, Winkler JB, Aneja M, Koch N, Fleischmann F, Pritsch K, Heller W, Stich S, Grams**
626 **TEE, Göttlein A, Matyssek R, Munch JC**. 2005. Short Term Effects of Ozone on the Plant-
627 Rhizosphere-Bulk Soil System of Young Beech Trees. Plant Biol **7**:728–736.

628 15. **Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E**. 1995. Patterns and regulation of
629 mycorrhizal plant and fungal diversity. Plant Soil **170**:47–62.

630 16. **Bossio DA, Scow KM**. 1995. Impact of carbon and flooding on the metabolic diversity of
631 microbial communities in soils. Appl Environ Microbiol **61**:4043–4050.

632 17. **Bossio DA, Scow KM, Gunapala N, Graham KJ**. 1998. Determinants of Soil Microbial
633 Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty
634 Acid Profiles. Microb Ecol **36**:1–12.

635 18. **Pettersson M, Bååth E**. 2003. Temperature-dependent changes in the soil bacterial community
636 in limed and unlimed soil. FEMS Microbiol Ecol **45**:13–21.

637 19. **Gschwendtner S, Leberecht M, Engel M, Kublik S, Dannenmann M, Polle A, Schloter M**. 2015.
638 Effects of Elevated Atmospheric CO2 on Microbial Community Structure at the Plant-Soil
639 Interface of Young Beech Trees (Fagus sylvatica L.) Grown at Two Sites with Contrasting Climatic
640 Conditions. Microb Ecol **69**:867–878.

641 20. **Geßler A, Schrempp S, Matzarakis A, Mayer H, Rennenberg H, Adams MA**. 2001. Radiation
642 modifies the effect of water availability on the carbon isotope composition of beech (Fagus
643 sylvatica). New Phytol **150**:653–664.

644 21. **Geßler A, Jung K, Gasche R, Papen H, Heidenfelder A, Börner E, Metzler B, Augustin S,**
645 **Hildebrand E, Rennenberg H**. 2005. Climate and forest management influence nitrogen balance
646 of European beech forests: microbial N transformations and inorganic N net uptake capacity of
647 mycorrhizal roots. Eur J For Res **124**:95–111.

648 22. **Rygiewicz PT, Johnson MG, Ganio LM, Tingey DT, Storm MJ**. 1997. Lifetime and temporal
649 occurrence of ectomycorrhizae on ponderosa pine (Pinus ponderosa Laws.) seedlings grown
650 under varied atmospheric CO2 and nitrogen levels. Plant Soil **189**:275–287.

651 23. **Valtanen K, Eissfeller V, Beyer F, Hertel D, Scheu S, Polle A**. 2014. Carbon and nitrogen fluxes
652 between beech and their ectomycorrhizal assemblage. Mycorrhiza **24**:645–650.

653 24. **Ellenberg H, Strutt G**. 2009. Vegetation ecology of Central Europe, 4th ed. Cambridge University
654 Press, Cambridge; New York.

655 25. **Rennenberg H, Dannenmann M, Geßler A, Kreuzwieser J, Simon J, Papen H**. 2009. Nitrogen
656 balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol
657 **11**:4–23.

658 26. **Dannenmann M, Gasche R, Ledebuhr A, Holst T, Mayer H, Papen H**. 2007. The effect of forest
659 management on trace gas exchange at the pedosphere–atmosphere interface in beech (Fagus
660 sylvatica L.) forests stocking on calcareous soils. Eur J For Res **126**:331–346.

661 27. **Bilela S, Dounavi A, Fussi B, Konnert M, Holst J, Mayer H, Rennenberg H, Simon J**. 2012.
662 Natural regeneration of Fagus sylvatica L. adapts with maturation to warmer and drier
663 microclimatic conditions. For Ecol Manag **275**:60–67.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

664 28. **Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Geßler A**. 2003. Carbon
665 and oxygen isotope composition of organic compounds in the phloem sap provides a short-term
666 measure for stomatal conductance of European beech (Fagus sylvatica L.). Plant Cell Environ
667 **26**:1157–1168.

668 29. **International Union of Soil Sciences Working Group WRB**. 2007. World Reference Base for Soil
669 Resources 2006, First Update 2007. Rome.

670 30. **Dannenmann M, Gasche R, Ledebuhr A, Papen H**. 2006. Effects of forest management on soil N
671 cycling in beech forests stocking on calcareous soils. Plant Soil **287**:279–300.

672 31. **Ritter E, Dalsgaard L, Einhorn KS**. 2005. Light, temperature and soil moisture regimes following
673 gap formation in a semi-natural beech-dominated forest in Denmark. For Ecol Manag **206**:15–
674 33.

675 32. **Dyckmans J**. 2000. Untersuchung der Aufnahme und Translokation von C und N in Buchen in
676 Abhängigkeit der atmosphärischen CO2-Konzentration und bauminternen N-Vorräte unter
677 Einsatz der stabilen Isotope ^{15}N und ^{13}C. Diss Georg-August-Univ Gött.

678 33. **Wu X, Yao Z, Brüggemann N, Shen ZY, Wolf B, Dannenmann M, Zheng X, Butterbach-Bahl K**.
679 2010. Effects of soil moisture and temperature on CO2 and CH4 soil–atmosphere exchange of
680 various land use/cover types in a semi-arid grassland in Inner Mongolia, China. Soil Biol
681 Biochem **42**:773–787.

682 34. **Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kögel-Knabner I, Knicker H, Mayer**
683 **H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H**. 2009. Tree girdling provides insight on
684 the role of labile carbon in nitrogen partitioning between soil microorganisms and adult
685 European beech. Soil Biol Biochem **41**:1622–1631.

686 35. **Guo C, Simon J, Gasche R, Naumann PS, Bimüller C, Pena R, Polle A, Kögel-Knabner I, Zeller B,**
687 **Rennenberg H, Dannenmann M**. 2013. Minor contribution of leaf litter to N nutrition of beech
688 (Fagus sylvatica) seedlings in a mountainous beech forest of Southern Germany. Plant Soil
689 **369**:657–668.

690 36. **Wu H, Dannenmann M, Fanselow N, Wolf B, Yao Z, Wu X, Brüggemann N, Zheng X, Han X,**
691 **Dittert K, Butterbach-Bahl K**. 2011. Feedback of grazing on gross rates of N mineralization and
692 inorganic N partitioning in steppe soils of Inner Mongolia. Plant Soil **340**:127–139.

693 37. **Perakis SS, Hedin LO**. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth
694 temperate forest, southern Chile. Ecology **82**:2245–2260.

695 38. **Agerer R**. 1987-2006. Colour Atlas of Ectomycorrhizae. Einhorn Verlag, Schwabisch-Gmünd.

696 39. **Gardes M, Bruns TD**. 1993. ITS primers with enhanced specificity for basidiomycetes -
697 application to the identification of mycorrhizae and rusts. Mol Ecol **2**:113–118.

698 40. **White TJ, Bruns T d, Lee S**. 1990. Amplification and direct sequencing of fungal ribosomal RNA
699 genes for phylogenetics. PCR Protoc Guide Methods Appl Eds MA Innis DH Gelfand JJ Sninsky TJ
700 White Acad Press N Y 315–322.

701 41. **Gschwendtner S, Reichmann M, Müller M, Radl V, Munch JC, Schloter M**. 2010. Effects of
702 genetically modified amylopectin-accumulating potato plants on the abundance of beneficial
703 and pathogenic microorganisms in the rhizosphere. Plant Soil **335**:413–422.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

704 42. **Hammer Ø, Harper DAT, Ryan PD**. 2001. PAST-Palaeontological statistics. Www Uv Es~
705 Pardomvpe20011pastpastprogpast Pdf Acessado Em **25**:2009.

706 43. **Keitel C, Matzarakis A, Rennenberg H, Gessler A**. 2006. Carbon isotopic composition and
707 oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (Fagus
708 sylvatica L.) along a climate gradient. Plant Cell Environ **29**:1492–1507.

709 44. **Jump AS, Hunt JM, Martínez-Izquierdo JA, Peñuelas J**. 2006. Natural selection and climate
710 change: temperature-linked spatial and temporal trends in gene frequency in Fagus sylvatica.
711 Mol Ecol **15**:3469–3480.

712 45. **Rose L, Leuschner C, Köckemann B, Buschmann H**. 2009. Are marginal beech (Fagus sylvatica L.)
713 provenances a source for drought tolerant ecotypes? Eur J For Res **128**:335–343.

714 46. **Pluess AR, Weber P**. 2012. Drought-Adaptation Potential in Fagus sylvatica: Linking Moisture
715 Availability with Genetic Diversity and Dendrochronology. PLoS ONE **7**:e33636.

716 47. **Weber P, Bugmann H, Pluess AR, Walthert L, Rigling A**. 2013. Drought response and changing
717 mean sensitivity of European beech close to the dry distribution limit. Trees **27**:171–181.

718 48. **Fotelli MN, Rienks M, Rennenberg H, Geßler A**. 2004. Climate and forest management affect
719 15N-uptake, N balance and biomass of European beech seedlings. Trees **18**:157–166.

720 49. **Stoelken G, Simon J, Ehlting B, Rennenberg H**. 2010. The presence of amino acids affects
721 inorganic N uptake in non-mycorrhizal seedlings of European beech (Fagus sylvatica). Tree
722 Physiol **30**:1118–1128.

723 50. **Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M**. 2009. Preferential allocation to
724 beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecol Lett **12**:13–21.

725 51. **Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR,**
726 **Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking**
727 **H**. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science
728 **333**:880–882.

729 52. **Koranda M, Schnecker J, Kaiser C, Fuchslueger L, Kitzler B, Stange CF, Sessitsch A, Zechmeister-**
730 **Boltenstern S, Richter A**. 2011. Microbial processes and community composition in the
731 rhizosphere of European beech – The influence of plant C exudates. Soil Biol Biochem **43**:551–
732 558.

733 53. **Zhou Z, Hogetsu T**. 2002. Subterranean community structure of ectomycorrhizal fungi under
734 Suillus grevillei sporocarps in a Larix kaempferi forest. New Phytol **154**:529–539.

735 54. **Guidot A, Debaud J-C, Effosse A, Marmeisse R**. 2004. Below-ground distribution and
736 persistence of an ectomycorrhizal fungus. New Phytol **161**:539–547.

737 55. **Treseder KK**. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and
738 atmospheric CO2 in field studies. New Phytol **164**:347–355.

739 56. **Geßler A, Keitel C, Nahm M, Rennenberg H**. 2004. Water shortage affects the water and
740 nitrogen balance in Central European beech forests. Plant Biol Stuttg Ger **6**:289–298.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

741 57. **Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE**. 2013. Climate
742 change may cause severe loss in the economic value of European forest land. Nat Clim Change
743 **3**:203–207.

744 58. **Bolte A, Czajkowski T, Kompa T**. 2007. The north-eastern distribution range of European
745 beech—a review. Forestry **80**:413–429.

746

747

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

748 **Table 1. Characteristics of root tips of young beech trees (*Fagus sylvatica*) originating from**
749 **dry (SW) and moist (NE) beech forests.** Trees from the natural regeneration collected on the
750 south west and the north east slope in beech forests were cultured for two months in a sand-peat
751 mixture and fertilized regularly. Trees were harvested regularly within the experimental week
752 (n = 8 per site and sampling date). Data show means ± SE of all sampling dates per site. P-
753 values of General Linear Models for the factor "Site" are shown, because the factor "Time" had
754 no significant effect ($P_{time} > 0.05$). * p value of the permutation test calculated with 1000
755 permutation matrices. P-values < 0.05 are highlighted in bold letters.

756

757

758		NE	SW	P_{site}
759				
760	Mycorrhizal root tips [%]	38.08 ± 2.38	30.36 ± 2.55	**0.03**
761	Species richness	32	36	**0.001***
762	Dead root tips [%]	6.57 ± 0.93	12.87 ± 1.94	**<0.001**
763	Non mycorrhizal root tips [%]	55.34 ± 2.51	56.77 ± 2.48	0.69

764

765

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

766 **Table 2: $\delta^{13}C$ signatures in plant tissues and ectomycorrhizas of young beech trees**
767 **(*Fagus sylvatica*) originating from dry (SW) and moist (NE) beech forests.** Trees from the
768 natural regeneration were cultured for two months in a sand-peat mixture, fertilized regularly
769 and exposed to $^{13}CO_2$ for two days. $\delta^{13}C$ was analyzed at day 0 and day 8 after the start of
770 labeling (n = 8 per site and sampling date). Data show means ± SE. P-values for a
771 multivariate ANOVA with site and time as fixed factors and for a one way ANOVA for the
772 comparisons of tissues are shown in columns and rows, respectively. Different letters indicate
773 significant differences (P < 0.05) of the tissues calculated post hoc with the HSD test. P-
774 values < 0.05 are highlighted in bold letters. FR = fine roots, EMF = ectomycorrhizal fungi,
775 NM = non-mycorrhizal roots

776

777

Site	Time	Leaf	FR	EMF	NM	$P_{(tissue)}$
		$\delta^{13}C$ (‰)				
NE	0	-31.3 ± 0.2a	-30.0 ± 0.3ab	-29.2 ± 0.5b	-30.7 ± 0.6a	**0.021**
NE	8	-30.3 ± 0.3a	-28.2 ± 0.3a	-27.0 ± 0.7a	-17.4 ± 1.9b	**<0.001**
SW	0	-32.0 ± 0.3a	-28.7 ± 0.6b	-29.9 ± 0.3b	-30.0 ± 0.6b	**0.002**
SW	8	-30.4 ± 0.2a	-27.3 ± 0.5b	-27.0 ± 0.9b	-16.3 ± 1.4c	**<0.001**
P_{Site}		0.123	**0.023**	0.571	0.482	
P_{Time}		**<0.001**	**0.002**	**0.001**	**<0.001**	
$I_{(Site\ x Time)}$		0.403	0.734	0.607	0.858	

778
779
780
781
782
783
784
785
786
787
788
789

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

790 **Figure legends**

791

792 **Figure 1. ^{15}N accumulation kinetics in soil N compounds, microbial biomass,**
793 **ectomycorrhizas, and plant tissues.** Natural beech regeneration was transplanted into a
794 sand-peat mixture, grown with regular fertilizer application for two months under identical
795 conditions and labeled for 8d with $^{15}NH_4^+$. Data are means (n = 8, ± SE) in atom-% excess
796 (APE) for ammonia (a), nitrate (b), DON (dissolved organic nitrogen, c) and microbial
797 biomass (d) in soil as well as for leaves (e), fine roots (f), non mycorrhizal root tips (g), and
798 mycorrhizal root tips (h). Note different scales. NE: closed circles, SW: open circles, Data
799 were compared by General Linear Models with "Time" and "Site" as fixed factors. P-values
800 for the factor "Site" are indicated in the figure. All P values for the factor "Time" were
801 significant at $P < 0.05$.

802

803 **Figure 2. Abundance of functional genes involved in the nitrogen cycle (a: nifH, b: amoA**
804 **AOB, c: narG, d: nirK) in the rhizosphere soil of trees originating from NE (grey) and**
805 **SW (white) forests.** Copy numbers of genes are shown before (day 0) and at day 1, 3, and
806 after labelling (n=8). Differences were considered to be significant when $p < 0.05$.

807

808 **Figure 3. Non-metric multidimensional scaling for ectomycorrhizal communities.** The
809 abundance based Morisita index was used as similarity measure to calculate a two-
810 dimensional NMDS based on the abundances of the ectomycorrhizal species. N (blue) and S
811 (red) refer to NE or SW treatments and 0, 1, 3, and 8 to the sampling dates where the
812 ectomycorrhizal species composition was determined. Information for the species
813 composition of ectomycorrhizal communities is shown in Table S1. Explanatory variables
814 (green vectors) were the frequencies of dead roots (Mort), ectomycorrhizal-colonized root tips
815 (EM), and non-mycorrhizal roots (NM), the frequencies of ascomycota, basidiomycota and

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM
Applied and Environmental Microbiology

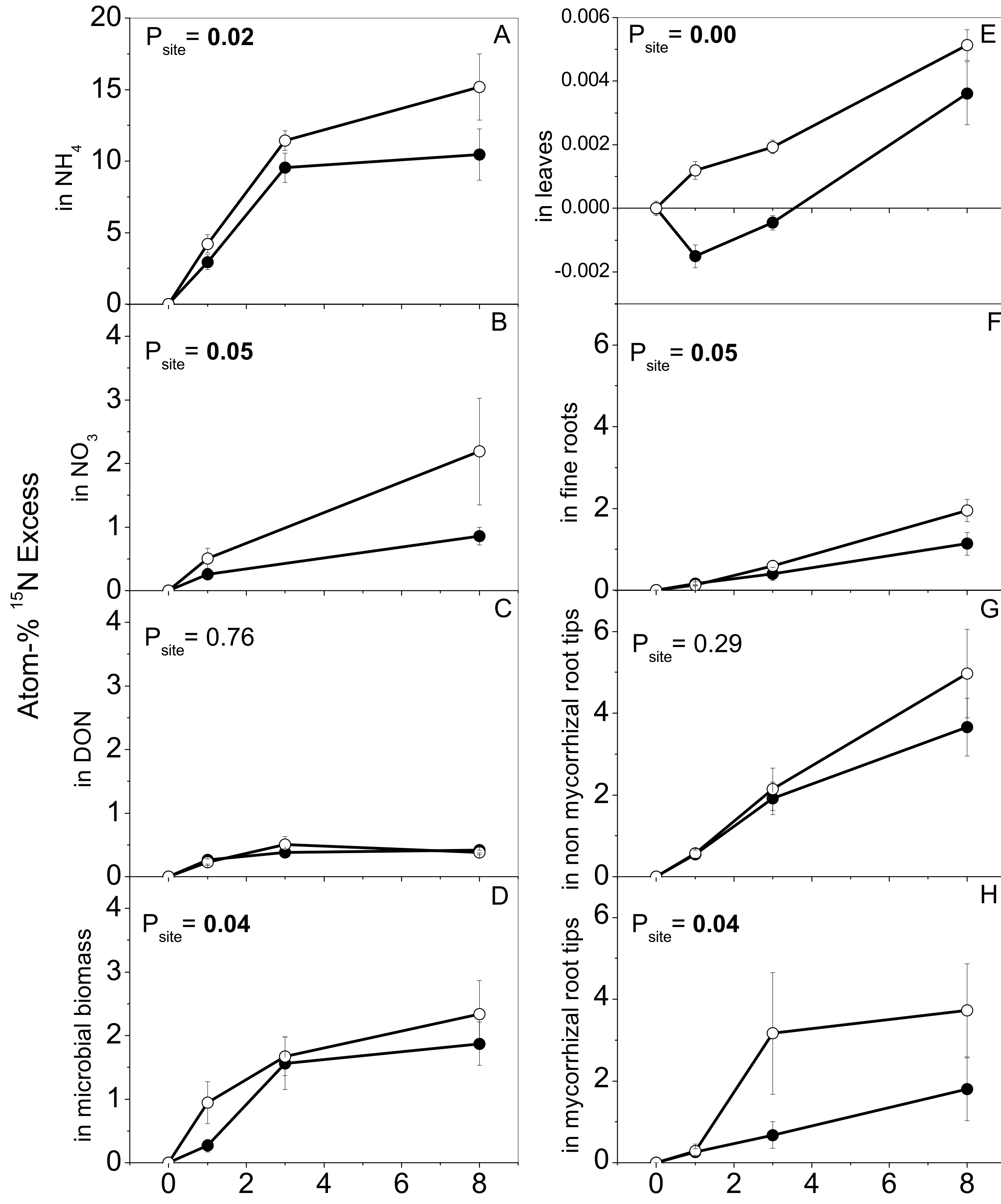
816 unknown fungal divisions, plant biomass (PBM), microbial biomass (MBN), plant ^{15}N uptake

817 (15Nup_plant) and microbial ^{15}N uptake (15Nup_MB). Stress: 0.215.

Accepted Manuscript Posted Online

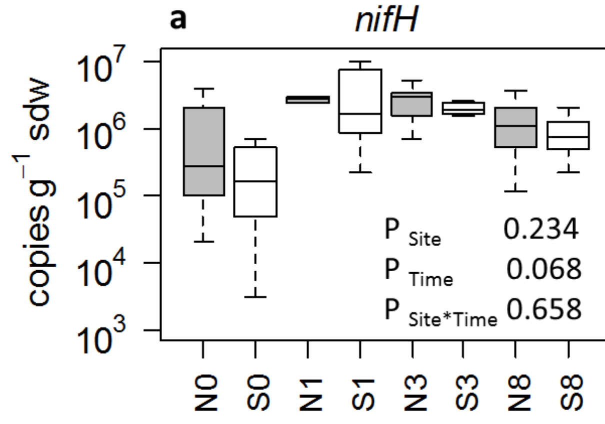
Applied and Environmental Microbiology

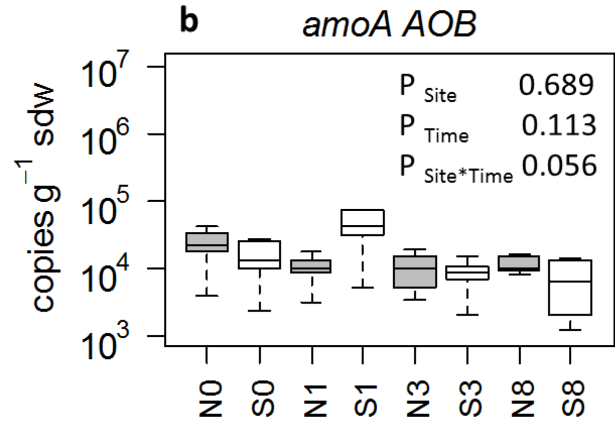
AEM

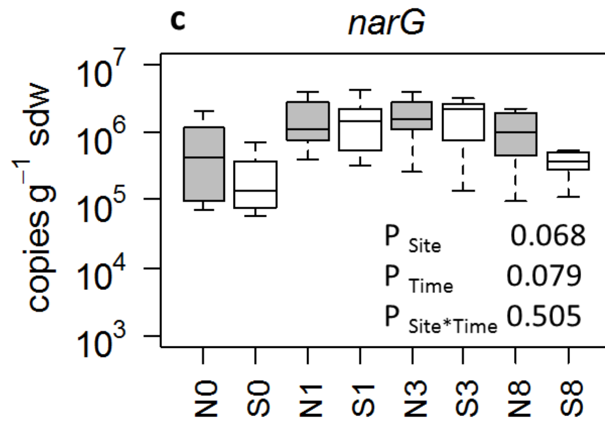


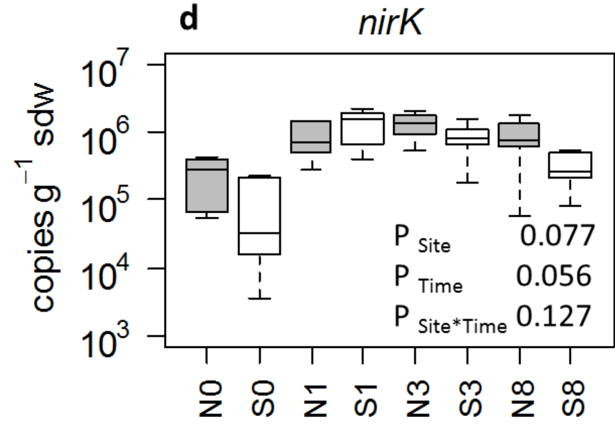
Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology









Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology