The effect of plant-derived carbon on rhizospheric microbial communities decomposing different litter types and nutrient return

Running title: Plant effects on nutrient cycling in the rhizosphere

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Abstract

Interactions between plants and microorganisms strongly affect ecosystem functioning as processes of plant productivity, litter decomposition and nutrient cycling are controlled by both organisms. Though two-sided interactions between plants and microorganisms and between microorganisms and litter decomposition are areas of major scientific research, our understanding of the three-sided interactions of plant-derived carbon flow into the soil microbial community and their follow-on effects on ecosystem processes like litter decomposition and plant nutrient acquisition remains limited. Therefore, we performed a microcosm experiment with two plant communities differing in their association with arbuscular mycorrhizal fungi (AMF). By applying a $^{13}CO_2$ pulse label to the plant communities and adding various ^{15}N labelled litter types to ingrowth cores, we simultaneously traced the flow of plant-derived carbon into soil microbial communities and the return of mineralized nitrogen back to the plant communities. We observed that net ^{13}C assimilation by the rhizospheric microbial communities and community composition depended on both, plant-AMF association and the litter type being decomposed. AMF-associated plant communities invested less ^{13}C into the litter decomposing soil microbial community but gained similar amounts of ^{15}N from litter decomposition compared to non-AMF plant communities. This effect was driven by significantly lower net ^{13}C assimilation per ^{15}N gain of saprotrophic fungi and bacterial, and significantly higher one of AM fungi compared to NM plant communities. Additionally the net ^{13}C assimilation of the rhizospheric microbial community from AMF-associated plant communities depended on litter type. Lowest ^{13}C assimilation was found for microbial and plant litter decomposition and highest for inorganic nitrogen. Large parts of the soil microbial community showed most interestingly redundant functions and different parts of the microbial community were dependent on the

plant-microorganism-litter context activated by plant-derived carbon in order to decompose specific litter types. Therefore, our results suggest that ecosystem processes like litter decomposition can only be fully understood if the whole plant-microorganism-litter context is investigated. Moreover our results give first hints that the plant AMF association might help to get less carbon costly access to nutrients locked in bacterial and plant necromass.

Introduction

Plant growth is mostly limited by the availability of nutrients, such as nitrogen (N) and phosphorous (P) in soil (Veresoglou et al., 2012). These nutrients are not readily available to plants as they are bound in soil organic matter and the mineral matrix. Consequently plants invest substantial parts of their assimilated carbon into soil via root exudates (sugars, amino acids and organic acids) and root growth associated cell debris, which stimulates microbial decomposition of soil organic matter and releases nutrients for plant and microbial reuse (Jacoby et al., 2017; Kuzyakov and Xu, 2013). However, immediate uptake of the released nutrients by plants is not always possible (Dijkstra et al., 2013) as the soil microbial communities themselves have a high nutrient demand and assimilate nutrients more efficiently than plants (Levin and Angert, 2015; Zhu et al., 2017). Since nutrient cycles in soil are almost entirely controlled by microorganisms, variations in plant exudation patterns and associations of plants and microorganism can have a tremendous impact on the rates of nutrient cycling (Craine et al., 2007; Veresoglou et al., 2012).

To adequately benefit from the microbial nutrient release some plants rely solely on their root systems while many others associate with specific soil microorganisms that colonize roots (Bonfante and Genre, 2010; Nuccio et al., 2013). A well-known association of this kind occurs between plants and arbuscular mycorrhizal fungi (AMF) (Davison et al., 2015). Though AMF do not decompose organic matter themselves (Tisserant et al., 2013), they colonize other saprophytic organisms that mineralize soil organic matter and compete with them for the nutrients at similar uptake rates (Bunn et al., 2019; Cheng et al., 2012; Thirkell et al., 2016). Plant support this process by altering their root exudate composition (Bonfante and Genre, 2010) and use the hyphal network to increase the soil volume that is available for nutrient uptake (Bitterlich et al., 2018). In contrast, non-mycorrhizal plants only release labile carbon to the microbial communities around their roots and lack the advantages of extended

soil volume accessibility and nutrient uptake efficiency (Karlowsky et al., 2018). As a result, they only benefit indirectly from decomposition related nutrient release. However, it remains unclear whether there are trade-offs for plants that depend on the type of belowground microbial associations and to what extent presence or absence of such microbial associations can affect decomposition related microbial processes.

For plants, the AMF-associated (AM) or non-mycorrhizal (NM) nutrient uptake strategies involve export of different amounts of carbon into microbial communities. AM plants are suggested to export more carbon to microbial communities than NM plants (Drigo et al., 2010; Scheffknecht et al., 2007) as the former supports an additional hyphal network (Cavagnaro et al., 2012). In comparison to NM plants this hyphal network supports the AM plants in multiple ways such as offering its roots greater soil coverage (Sanders and Tinker, 1973), access to immobile nutrient pools (Cavagnaro et al., 2012) and access to distant water pockets (16, 21). Hence, the overall plant carbon allocation into the root systems and the carbon export to associated microbial processes vary depending on the nutrient uptake strategy, which was successfully demonstrated using mesh guarded ingrowth cores (Nuccio et al., 2013; Throckmorton et al., 2012). However, it remains unclear if carbon intensive specific mutualistic associations like with AMF also result in higher carbon costs per nutrient return and if the net plant carbon uptake by decomposing microorganisms is directly related to the plant nutrient uptake.

The microbial decomposition process itself is well understood. Microbial growth and activity in soil is primarily limited by the availability of labile carbon which is either released via plant exudation or decomposition of plant litter and soil organic matter (Hütsch et al., 2002). Both quantity of labile carbon inputs (de Graaff et al., 2010) and alteration of biochemical profile of root exudates by AMF infection (Shi et al., 2016; Zhalnina et al., 2018) can alter activity and composition of microbial communities associated with the rhizosphere and litter decomposition (Cheng et al., 2012; Nottingham et al., 2013; Nuccio et al., 2013).

107 Similarly, interactions between litter quality and decomposer community are also well known
108 and suggest that the amount and form of nitrogen play an important role in the extent of litter
109 decomposition and nitrogen uptake by the plants (Schimel and Bennett, 2004). So far it was
110 considered that bacterial members of microbial community favor nitrogen rich substrates
111 whereas fungal members favor carbon rich lignocellulosic substrates during decomposition
112 (Güsewell and Gessner, 2009; Meier et al., 2015; Strickland et al., 2009). Recent studies,
113 however, have demonstrated that many bacteria also decompose lignocellulose-rich substrates
114 (López-Mondéjar et al., 2016) and that the soil microbial community is in general
115 characterized by a high functional redundancy (Louca et al., 2018).

116 Soil receives nitrogen containing substances either via carbon rich plant residues, nitrogen
117 rich microbial necromass or inorganic nitrogen from deposition and fertilizers (Gleixner,
118 2013; Liang et al., 2017; Thuille et al., 2015). However, these nitrogen inputs differ in their
119 biochemical properties (litter type) and carbon to nitrogen ratios (litter quality), thus
120 confounding the effects that plant communities have on the decomposer communities in soil.
121 Under natural conditions, the decomposer community is shaped by litter characteristics and
122 their physiological boundaries, which are defined by their energetic and nutritional demands
123 (Thomson et al., 2013). Hence, by exposing rhizospheric microbial communities to litter of
124 identical quality but different types, a better understanding of the mechanisms by which
125 plants affect the decomposer community can be obtained.

126 In summary, large uncertainties still exist regarding the mechanistic benefits of plant-
127 microbial associations with respect to litter decomposition and nutrient return. Therefore, we
128 assembled two plant communities that differed in their ability to establish an AMF-based
129 mycorrhizal association (AM or NM) and amended the soil inside the root ingrowth cores
130 with various ^{15}N labeled litter types (plant root litter, microbial necromass and inorganic N) of
131 similar quality (C/N ratio) and traced the net plant derived carbon flow into the microbial
132 community (PLFA) using a $^{13}CO_2$ pulse label experiment and investigated the associated

133 microbial community structure (DNA and RNA). We first hypothesized that AM associations
134 result in greater plant carbon export to the microbial community (including AMF) for
135 nitrogen mineralization. We also hypothesized that carbon cost per nutrient gain is higher in
136 AM association than in NM association, since AM associations require a considerable amount
137 of plant carbon for maintaining the hyphal network. We finally hypothesize that the
138 developed soil microbial community depends in the first order on the plant community (AM
139 vs NM) and its carbon export, and in the second order on the litter type independent from its
140 quality.

141 Materials and Methods

142 Microcosm Experiment

143 We set up microcosms (19 x 12.5 x 9.5 cm) and established mixed temperate plant
144 communities of two types namely AMF dependent plant community (*Linum ussitatissimum,*
145 *Linum perenne, Festuca rubra* and *Tagetes patula*) (n = 72 pots) termed as the AM plant
146 community and non-mycorrhizal community (*Dianthus caryophyllus*, *Matthiola longipetala*
147 *sub-species bicornis*, *Dianthus barbaratus* and *Carex arenaria*) (n = 36 pots) termed as NM
148 plant community (Brundrett, 2015b, 2015a). The soil used for the experiment was collected
149 from The Jena Experiment (Roscher et al., 2004) located in Jena, Germany in March 2015.
150 After removing the root mat in upper 2 cm, the top 5 cm of soil was collected, sieved (<2
151 mm) and all remaining roots were removed. The soil was stored at 4 ^{o}C until the
152 establishment of the microcosms

153 Each microcosm unit had a central PVC ingrowth core (diameter = 5 cm) with two
154 tiers of 8 windows (4 x 1.5 cm) each guarded by a mesh (Figure 1). The mesh with a 1000
155 micron pore size allowed entry of fine roots and hyphae and the one with 30 micron pore size
156 allowed entry of AMF hyphae only. The microcosms and the ingrowth cores were filled with
157 soil and pre-equilibrated for 2 weeks in the greenhouse. Plant saplings that had developed the

158 first true leaves were planted with equal evenness into the pots excluding the ingrowth core.
159 The microcosms were randomly distributed in the greenhouse and incubated for 4 months
160 until the plants were fully developed. Irrigation was provided using an ebb and flood system
161 for 5 min daily and lighting for the plants (Son- T Agro 430 W HPS bulbs, primary light
162 range = 520–610 nm, Philips Lighting Company, Somerset, NJ, USA) was provided daily for
163 12 h.

164 For litter amendment to the fully developed microcosms, the central ingrowth core
165 was removed. The soil was sieved (<2mm) to remove fine roots. 7.5, 0.8 & 0.012 g of ^{15}N
166 labelled plant root litter, microbial necromass and inorganic nitrogen were respectively added
167 to 200 g of sieved soil (Figure 1). An additional amount of 1.28 and 1.58 g of xanthan gum
168 (Sigma-Aldrich, Inc) was added to the microbial necromass and inorganic nitrogen treatment
169 respectively to keep the carbon and nitrogen inputs across all amendments (avg. C:N = 46 ±
170 1.1 s.e.m.) similar. Overall, the amendments were kept small, the total carbon and nitrogen
171 content of the ingrowth core soil was increased by ca. 0.15 % and ca. 0.01 %, respectively.
172 The amended soil was mixed, refilled into the ingrowth core and replaced into the
173 microcosms (Figure 1).

174 2 weeks after the amendment, which allowed roots and hyphae to regrow into the
175 ingrowth core, the microcosms were exposed to a $^{13}CO_2$ pulse in an air tight plexiglass
176 chamber (Malik et al., 2015). For each litter amendment, all pots (n=27) were subjected to a
177 $^{13}CO_2$ pulse together in a well-lit (PAR intensity >500 µmol photons $m^{-2}s^{-1}$) chamber (volume
178 = 0.15 m^3). The chamber air was flushed with CO_2 free synthetic air until the CO_2
179 concentration fell below 150 ppm. Enriched $^{13}CO_2$ (99 atom %) was introduced into the
180 chamber using a mass flow controller (model- MF1-20sccm-N2, MKS Instruments Inc.) at a
181 flow rate of 3.33 x 10^{-7} m^3s^{-1} for 10 min and circulated using fans. The CO_2 concentration was
182 monitored online by a cavity ringdown spectrometer (G2101-I, Picarro Inc., Santa Clara, CA,
183 USA) throughout the labeling period of 1h. An additional maintenance stream of $^{13}CO_2$ was

184 introduced halfway at a flow rate of 1.67 x 10^{-7} m^3s^{-1} for 5 min that kept the chamber CO_2
185 concentration between 350 ppm and 410 ppm.

186 At the end of the labelling period the pots were removed from the chamber and
187 returned back to the greenhouse and destructively harvested at 0 h, 4 h, 24 h and 240 h from
188 the pulse. Soil from the ingrowth compartments were sieved (<2 mm) and divided into three
189 sub-samples and stored at -80 ºC for molecular analysis within 1 h of harvest, dried at 105 ºC
190 for 48 h and stored at room temperature for elemental analysis. The above and below ground
191 plant tissues were collected at the last destructive harvest (240 h) and dried at 60 ºC for 48 h
192 and subsequently stored for future analysis. Control pots for each litter amendment (n=9)
193 were harvested prior to the labeling in order to avoid cross contamination from ^{13}C labeled
194 air.

195

196 **Production of labeled litter**

197 To follow nutrients released by decomposition to the plant tissues, ^{15}N labelled
198 organic matter of three types namely plant root litter, microbial necromass and synthetic
199 substrate were used in this study. Plant root litter was harvested from perennial grass *Holcus*
200 *lanatus* grown in a pot (57x 37 x 20 cm) containing sand in a plant growth chamber that was
201 lit daily for 12 h at day temperatures of 23 °C and at 63 % rel. humidity. The plants were
202 watered 2-3 times a week and fertilized weekly with a balanced N free Hoagland's solution
203 amended with 0.40 atom% $K^{15}NO_3$ as the sole nitrogen source. At the end of 4 weeks the
204 plants were harvested and roots were cleaned with distilled water, dried and shredded to
205 remove effects of particle size.

206 Inoculum for producing microbial necromass was produced by slow centrifugation of
207 6 g of Jena Experiment soil in 20 ml of distilled water at 650 g for 15 min. The supernatant
208 was inoculated into M9 minimal medium amended with 20% glucose and contained $K^{15}NO_3$

209 (0.40 atom%) as only nitrogen source. The culture grew for 7 days at 19 °C on a horizontal
210 shaker. The entire cell mass was harvested by centrifugation (@ 3500 g for 15 mins) and re-
211 suspended in 10 ml of sterile distilled water and sterilized by autoclaving. The sterile pellet
212 was freeze dried for further use.

213 The inorganic nitrogen amendments were generated by mixing KNO_3 and $K^{15}NO_3$
214 such that the mixture had an isotopic signature of 0.40 atom % and used directly for the
215 amendments.

216 **Elemental and isotopic analysis of plant samples**

217 The shoot samples that were harvested at the end of experiment were subjected to
218 carbon and nitrogen elemental and isotope analysis. Briefly, oven dried shoot and root
219 biomass was ball-milled to a fine powder and weighed into tin capsules. Separate samples
220 were prepared for bulk ^{13}C and bulk ^{15}N analysis. The samples were analyzed using an
221 elemental analyzer EA1100 (CE, Mainland) coupled via a conflow III interface to a DELTA+
222 (Finnigan MAT) isotope ratio mass spectrometer (Malik et al., 2015).

223 **Biomarker extraction, quantification and compound specific isotope analysis**

224 To assess the ^{13}C flow into the microbial community, fatty acids from neutral lipids
225 (NL) and phospholipids (PL) were extracted from ingrowth core soil sampled at 0 h and 240
226 h. All biological replicates of each litter-rhizosphere combination that were collected at the
227 same time point were pooled and homogenized to create a composite sample from which
228 always 5g aliquots of soil were used for lipid extraction. We applied a modified Bligh and
229 Dyer (1959) lipid extraction protocol using pressurized solvent extraction, as described earlier
230 Karlowsky et al., (2018). The concentration and compound specific ^{13}C incorporated relative
231 to an unlabeled sample for bacterial PLFA markers (14:0, 15:0, 16:0, 17:0, 18:0, 14:0i, 15:0i,
232 15:0a, 16:0i, 17:0i, 17:0a, 16:0(10Me), 17:0(10Me), 18:0(10Me), 17:0cy, 19:0cy, 15:1,
233 16:1ω7, 16:1ω5, 16:1, 17:1, 18:1ω7 and 18:1ω9) and fungal PLFA (18:2ω6,9) was

234 determined using a gas chromatograph coupled with a flame ionization detector (FID) and
235 isotope ratio mass spectrometer (IRMS) respectively (Karlowsky et al., 2018). Additionally,
236 the NLFA biomarker 16:1ω5 was used as the marker for AMF.

237 The amount of ^{13}C incorporated was determined from samples collected after the pulse
238 minus the isotope content of unlabeled soil collected before the pulse (Karlowsky et al.,
239 2018). The amounts of saprotrophic fungal markers (PLFA - 18:2ω6,9) were converted into
240 biomass C values by applying conversion factor 11.8 nmol = 1 mg C (Klamer, 2004), for
241 bacterial biomarkers by applying conversion factor 363.3 nmol = 1mg C (Frostegård and
242 Bååth, 1996) and for 16:1ω5 NLFA by applying conversion factor 1.047 nmol = 1μg C
243 (Williams et al., 2017). The amount of ^{13}C in the specific biomarker was determined from the
244 C pool size of the biomarker corrected for soil weight and moisture (Karlowsky et al., 2018).
245 The total amount of ^{13}C in the bacterial and fungal biomarkers was used as a proxy for
246 microbial biomass that incorporated freshly fixed plant C. Net ^{13}C assimilated by soil
247 microbial community and its parts (saprophytic fungi, AMF and free-living bacteria) for
248 every unit of ^{15}N gained by the plant was used as an estimate of net plant C allocation for
249 nutrient gain to specific microbial groups.

250 **DNA and RNA based microbial community profiling**

251 To ascertain the microbial community composition, 1.09 ± 0.01 g (n = 27) of soil was
252 used to co-extract total RNA and DNA using the MoBio RNA Powersoil kit. The samples
253 were bead-beaten for one cycle at 5.5 m/s for 30 s with a FastPrep instrument (MP
254 Biomedicals Germany GmbH). DNA was extracted using a MoBio RNA Powersoil DNA
255 elution accessory kit. Traces of DNA that was co-extracted in the final RNA elution was
256 removed using a MoBio DNase Max kit. The purified DNA and RNA concentrations were
257 measured using Qubit 4 fluorometer (Thermo Fisher Scientific) using the broad range assay
258 kits for DNA and RNA. Additionally, the purity of the samples was checked using a nanodrop

259 spectrometer 2000c (Thermo Fisher Scientific) and all samples were found to have A260/280
260 and A260/230 ratios of 2 suggesting no protein or organic compound contamination. The
261 integrity of the RNA samples was visually verified using 0.8 % agarose gel electrophoresis.
262 Presence of sharp well-defined ribosomal RNA bands and smear in between and above these
263 bands was considered as an indicator of high integrity of the RNA. 200 ng of total RNA
264 extracted was converted into cDNA using Superscript^{TM} VILO kit (Thermo Fisher Scientific)
265 using random hexamers to ensure uniform conversion of all the RNA fragments. The DNA
266 and cDNA samples were further used for amplifying the 16S rRNA gene and ITS regions
267 respectively. Briefly, for the 16S rRNA gene sequencing, amplicon libraries were prepared
268 with V3-V5 primers (Haas et al., 2011) using PCR conditions described by Kozich et al.,
269 2013. ITS2 gene libraries were constructed by amplifying ITS region 2 using the fITS7
270 (forward) and ITS4 (reverse) primers (Ihrmark et al., 2012) with previously described PCR
271 conditions (Gweon et al., 2015). Both sets of primers also consisted of an appropriate
272 Illumina adapter, an 8-nt index sequence, a 10-nt pad sequence, a 2-nt linker (Kozich et al.,
273 2013). The amplicons were sequenced using Illumina MiSeq, v3 chemistry, 600 cycles.

274 **Processing of sequencing data**

275 For the bacterial community 16S rRNA gene sequencing data, the forward and reverse
276 reads obtained were merged using PEAR (v 0.9.6) with default parameters (Zhang et al.,
277 2014). The bacterial community composition was analyzed using the QIIME (1.8.0) pipeline
278 (Caporaso et al., 2010). Representative OTU's were picked using UCLUST using de novo
279 method at 97% similarity level and were assigned taxonomy using RDP classifier method
280 with the SILVA reference data set (release 132 for QIIME) (Edgar, 2010; Wang et al., 2007).
281 Total number of sequencing reads classified within the OTU table for the samples varied
282 between 4000 to 21982 reads. In order to make inter-sample comparisons OTU table obtained

283 was rarefied to 4000 sequences per sample using QIIME command single_rarefraction.py
284 (Caporaso et al., 2010).

285 Since fungal spores are ubiquitous, the ITS2 regions that were transcribed into RNA
286 were used to sample transcriptionally active fungi (Anderson and Parkin, 2007). The
287 amplified ITS region from total cDNA samples were used for sequencing. The amplified ITS
288 sequencing data was analyzed using the PIPITS pipeline using the default parameters (Gweon
289 et al., 2015). Briefly, the paired end sequences were merged using VSEARCH after which the
290 ITS2 region was extracted using ITSx after which they are matched to a trained UNITE
291 database at 97% similarity using RDP classifier to obtain an OTU table (Bengtsson-Palme et
292 al., 2013; Rognes et al., 2016). The OTUs detected were then assigned to fungal guilds using
293 FUNguild assignment app (http://www.stbates.org/guilds/app.php) (Nguyen et al., 2016).

294 The bacterial and fungal amplicon sequencing data and the associated metadata is
295 publicly available through the sequence read archive project number: SRP132171.

296

297 **Statistical data analysis**

298 One-way ANOVA was used to determine statistical differences amongst rhizosphere
299 types (AM vs NM) for root:shoot ratio and net microbial ^{13}C assimilation into total microbial
300 biomass. Two way ANOVA was applied to identify statistically significant differences due to
301 rhizosphere and litter types for ^{15}N assimilated by plant shoot and plant carbon allocation per
302 unit ^{15}N gain. All two-way ANOVA models that showed significant interactions of
303 rhizosphere-litter types were subject to Tukey's post-hoc test to identify differences amongst
304 litter types nested within rhizosphere types. Subsampled OTU tables that were binned to
305 different taxonomic groups at the phylum and class levels (bacteria) whereas fungal OTUs
306 were binned into guilds to compare effect of AM vs NM plant communities and litter
307 amendments on microbial (bacterial and fungal) community composition. The effects of litter

308 and rhizosphere type on microbial groups was separately determined by comparison of the F
309 values generated by a two-way ANOVA of their relative abundance. Differences between
310 microbial (bacterial and fungal) community composition affected by the experimental
311 treatment were determined using non-parametric PERMANOVA ("adonis" function in the
312 vegan package 2.5-2) (Oksanen et al., 2018). The dissimilarity based upon microbial
313 community composition due to litter and rhizosphere treatments were visualized as an NMDS
314 ordination of Bray-Curtis dissimilarity coefficients on a Hellinger transformed OTU table so
315 as to obtain the least amount of stress in the ordination (Ramette, 2007). All analysis was
316 performed using R software (v 3.4.3) with visualization being done with ggplot2 package
317 (Wickham, 2011).

318 # Results

319 ## Carbon allocation and Nitrogen return

320 The shoot biomass of the AM plant community was smaller (4.66 ± 0.17 g, n = 18)
321 compared to NM the plant community (7.42 ± 0.44 g, n = 9) but both had similar root
322 biomass ($F_{1,25}$ = 0.32; p = 0.582), which resulted in a higher root to shoot ratio for the AM
323 plant community. The AM plant community also allocated more carbon into their roots,
324 which was reflected in the root to shoot ratio of ^{13}C incorporated into the AM plant
325 community compared to the NM plant community ($F_{1,16}$ = 10.11, p = 0.005; Figure 2A).
326 Unexpectedly, the net ^{13}C assimilation of the rhizospheric microbial communities from the
327 AM plant community was significantly lower than that of the NM plant community ($F_{1,16}$ =
328 21.75, p < 0.001, Figure 2B).
329 The AM plant community gained 93.1 ± 4.7 μg ^{15}N from litter decomposition, which was not
330 significantly different from the NM plant community that gained 82.3 ± 10.7 μg ^{15}N (($F_{1,16}$ =
331 0.36, p = 0.558, Figure 3A). Neither the different litter types (AM: $F_{2,15}$ = 0.31, p = 0.743;
332 NM: $F_{2,6}$ = 0.99, p = 0.424) nor the mesh sizes of the ingrowth cores (%N - $F_{1,16}$ = 1.19, p =

333 0.290; ^{15}N - $F_{1,16} = 0.25$, $p = 0.623$) had a significant influence on the ^{15}N return into both
334 plant communities. Consequently, the AM plant community invested 66% less ^{13}C into the
335 microbial community per ^{15}N gain than the NM plant community ($F_{1,16} = 14.92$, $p = 0.002$;
336 Figure 3B). Moreover, the AM plant community allocated significantly more ^{13}C per ^{15}N
337 gained to AMF biomass ($F_{1,16} = 30.53$, $p < 0.001$; Figure 4A) whereas NM plant communities
338 allocated significantly more ^{13}C per ^{15}N gained into saprophytic fungi ($F_{1,16} = 15.47$, $p =$
339 0.001; Figure 4B) and bacteria ($F_{1,16} = 4.78$, $p=0.044$). Unexpectedly the amount of ^{13}C
340 allocated per ^{15}N gained depended only in the AM plant community on the type of
341 decomposed litter (litter – $F_{2,15} = 7.13$, $p = 0.009$, Figure 4). Specifically, in the AM
342 rhizosphere, colonization of inorganic N amended soil resulted in significantly more plant-
343 derived ^{13}C to be allocated to bacteria and saprotrophic fungi compared to microbial
344 necromass and plant litter amended soil (Figure 4B, C). Such litter-based differences were
345 missing in the NM rhizosphere.
346

347 **Microbial community composition**

348 Most individual microbial groups were responsive to either rhizosphere type (NM or
349 AM) or litter type (root litter or microbial necromass or inorganic N) (Figure 5). Litter type
350 strongly affected bacterial OTUs binned under *Bacteroidetes*, *Gemmamonadetes*, *Nitrospira*
351 and *Deltaproteobacteria* whereas amongst fungal guilds saprotrophic and ectomycorrhizal
352 guilds responded to litter types (Figure 5A). The bacterial groups affected by rhizosphere type
353 belonged to the *Acidobacteria*, *Proteobacteria* and *Betaproteobacteria* whereas amongst the
354 fungal guilds AMF responded the most to rhizosphere type (Figure 5B). Such responsiveness
355 of specific microbial groups to rhizosphere and litter type significantly affected the microbial
356 community composition (Figure 6 & Table 1). Expectedly, the fungal communities varied
357 strongly along the expected lines between both plant communities (Figure 6A, Table 1). This

358 was mainly explained by the higher abundance of AMF (Figure 6C). Litter amendments also
359 significantly affected the fungal community (Table 1). The bacterial community was also
360 influenced by both rhizosphere and litter type (Figure 6D & 6E; Table 1). The rhizosphere of
361 the NM plant community was dominated by phylum *Acidobacteria* whereas for AM plant
362 communities were dominated by Proteobacterial classes (esp. *Betaproteobacteria*) (Figure
363 6F). Specifically, root litter amendments also resulted in dominance of phylum
364 *Proteobacteria* whereas inorganic N amendments resulted in increased relative abundance of
365 phylum *Bacteroidetes* (Figure 6F). OTUs grouped under *Deltaproteobacteria* were in general
366 responsive to all litter amendments made in this study (Figure 6F).

367 Discussion

368 In this study we investigated how plant communities, differing in their association
369 with AMF, affected the carbon allocation to the microbial communities and the return of
370 mineralized nitrogen from different litter types. By combining a dual stable isotope labelling
371 with a sequencing analyses and compound specific isotope analysis of the soil microbial
372 community we found that AMF associated (AM) plant communities did not gain significantly
373 more nitrogen from litter decomposition than non-mycorrhizal (NM) plant communities.
374 However, AM plant communities had to invest less carbon into the soil microbial
375 communities, resulting in a significantly lower carbon per nitrogen cost than NM plant
376 communities (Figure 7). Even though AM plant communities foster a carbon demanding
377 fungal network, they benefit strongly from the AMF-targeted access to the decomposition
378 sites where plant-derived carbon is used to stimulate selected microorganisms for litter
379 decomposition. These findings are in line with previous studies showing the importance of the
380 AMF hyphal network on the associated soil bacterial community (Drigo et al., 2010;
381 Vestergård et al., 2008). The close interaction of AMF and bacteria potentially increases
382 decomposition efficiency and makes in the long run more nutrients available to the plant

383 communities. NM plant communities likely miss the targeted growth of AMF hyphae and
384 relay on untargeted saprophytic fungi and bacteria for decomposition, which increases the
385 overall carbon per nitrogen cost.

386 Furthermore, our results also showed that the net assimilation of plant carbon by the
387 rhizosphere microbial community depended on the biochemical characteristics of the litter
388 being decomposed. This suggests strongly that litter chemistry (litter type) is as important as
389 litter quality in the decomposition process and can modulate the extent of plant-derived
390 carbon required for decomposition (Jones et al., 2004). AMF-associated rhizospheric
391 microbial communities generally assimilated less plant carbon than the microbial
392 communities with NM plant communities. This suggests AMF allocated less labile C to the
393 free-living soil microbial community in order to take up mineralized N from decomposition.
394 The different net assimilation of the free-living microbial community from AM and NM plant
395 communities also suggests that the decomposition of different litter types strongly depended
396 on the association with AMF. This confirms that AMF indeed act as target oriented root
397 extensions that more effectively feed plant-derived carbon to the free-living microbial
398 community. Moreover, in response to the litter biochemistry, and thus the quantity of plant
399 available nutrients, plants and AMF likely control the amounts of exudates released. In turn,
400 the significant response of specific microbial groups to either the litter type and rhizosphere
401 type as well as the shift in the microbial community composition suggests that in addition to
402 the litter nutrient content (Stempfhuber et al., 2016) the interplay of litter type with plant
403 carbon inputs shapes microbial communities in the rhizosphere. Hence the composition of the
404 microbial decomposer community is adjusted to plant exudation and type of the litter to be
405 decomposed (Figure 7), making plant communities an important part of the decomposition
406 process (McLaren and Turkington, 2011). Expectedly, the fungal community primarily
407 differed between AM or NM plant communities, emphasizing the fungal role in
408 decomposition and nutrient gathering (Bukovská et al., 2018; Meier et al., 2015). However, in

409 the presence of AMF, we observed lower plant carbon assimilation by the free living
410 microbial community (Mueller et al., 2014), which was dominated by the bacterial
411 community. This suggests that primary decomposers in the AM rhizosphere were bacteria that
412 were fed and adapted to litter types by the AMF (Herman et al., 2012; Nuccio et al., 2013).
413 Most interestingly our results indicate for the first time that AM plant communities strongly
414 benefit from the association with AMF during microbial litter decomposition. AMF
415 assimilated more ^{13}C from plants but invested less carbon into the bacterial community for
416 decomposition. This might indicated that AMF, which have no capability to decomposed
417 lignocellulose structures of plant cell walls themselves (Tisserant et al., 2013), might be able
418 to decomposed peptidoglycan structures of microbial cell walls. This idea would be supported
419 by the dramatic up-regulation of genes encoding for enzymes containing a peptidoglycan
420 binding LysM domain and glycan hydrolases like lysozyme, glucosamidase and alpha
421 amylase that would be necessary for microbial cell wall decomposition (Tisserant et al., 2013;
422 Gude et al., 2012). If we consider that large parts of soil organic matter is microbial
423 necromass (Gleixner, 2013) the association of plants with AMF gain a new overall
424 importance for ecosystem functioning. However, additional experiments are needed to
425 confirm the new role of plant-AMF associations for microbial cell wall decomposition.
426

427 # Conclusion
428

429 Our results suggest that three way interactions between the plant community, the
430 microbial community and the decomposing litter type have fundamental importance for
431 ecosystem functioning. Especially nutrient recycling in the plant-microorganism-soil
432 continuum is shaped by these interactions leading to a plant-driven and context-dependent
433 stimulation of decomposers. Their decomposition capabilities are likely highly redundant as

biochemically identical litter is decomposed by different parts of the microbial community. In consequence, litter decomposition and nutrient recycling can only be fully understood if the whole continuum is investigated. Moreover, our results demonstrate for the first time that plant nutrient gain via symbiotic AMF is less carbon intensive and likely more targeted then untargeted exudation od NM plant communities. This might explain why a large part of the plant community relay on the AM nutrient uptake strategy.

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Author contributions

SC, GG and AM designed the study. SC and JH performed the experiment. SC performed the extractions and stable isotope analyses. TG and RG completed the downstream processing of nucleic acids, sequencing and supervised bioinformatics analysis. SC and ML completed the statistical analysis. SC, ML and GG conceptualized the work and wrote the manuscript and all authors contributed to revisions.

Conflict of Interest

The authors declare no conflict of interest

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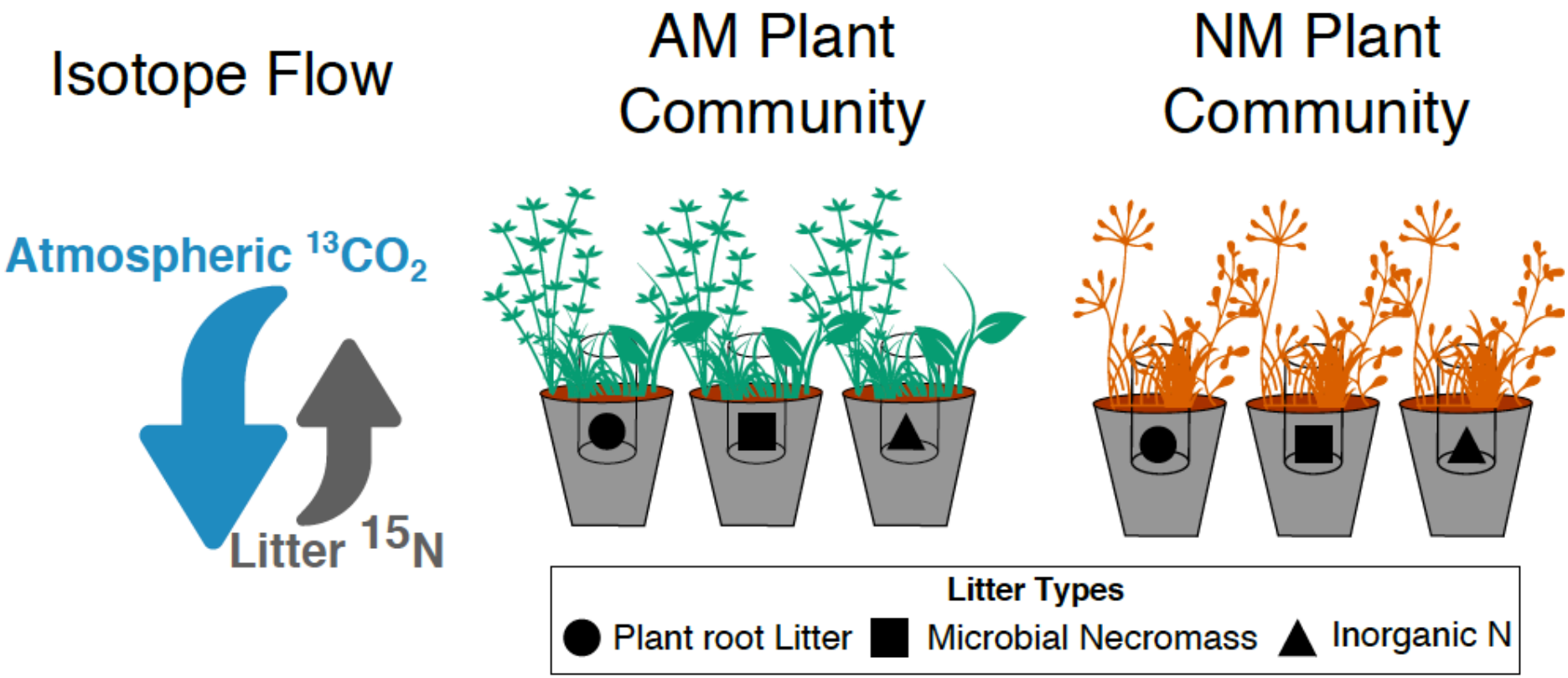
Figures and Tables Legends

Figure 1: Experimental setup of the study.



676 **Figure 2: Photosynthetically fixed carbon assimilated by plants and associated microbial**
677 **community. A:** Comparison of ratio of root to shoot ^{13}C content between AM (n = 9) and
678 NM (n = 9) the plant community types. Height of the bar represents the mean and error bars
679 represent standard error of mean. Different letters on bars designate significance differences.
680 **B:** Mean ^{13}C (ng) excess in soil microbial biomass of each plant community types. Colors
681 represent plant community types where Dark green=AM plant community and Orange= NM
682 plant community. Bars sharing the same letter are not significantly different.
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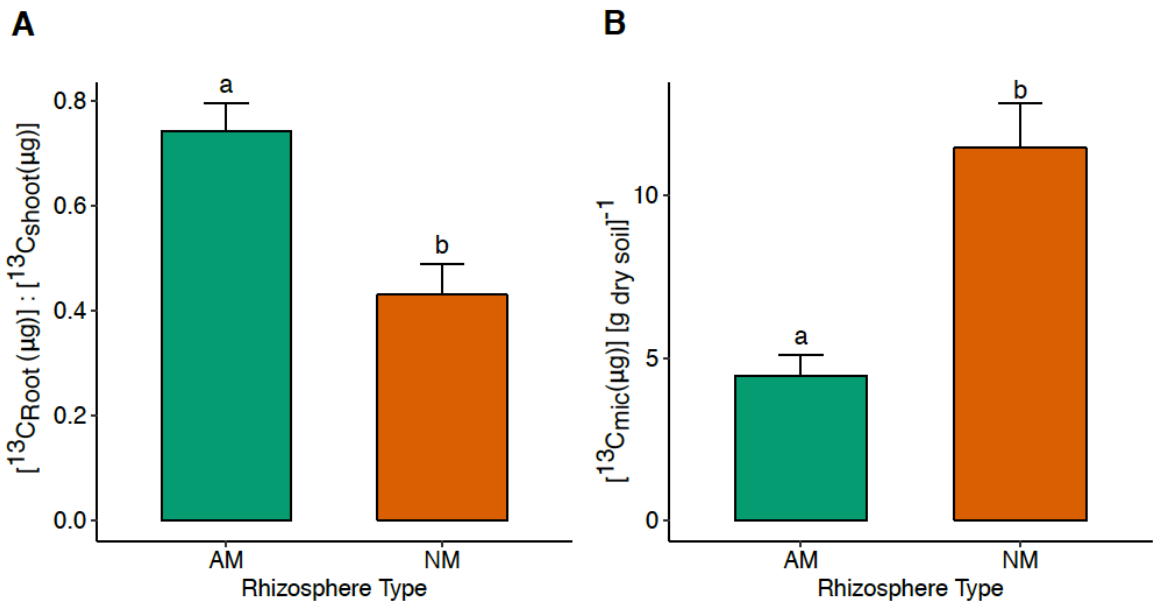

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Figure 3: Carbon investment into microbial community and return of decomposition released nitrogen. A: ^{15}N content (µg) of shoot biomass compared between AM (n = 9) and NM (n = 9) plant community (AM – Dark green and NM - Orange). Horizontal bars represent means for each group and error bars represent standard error of mean (n=9 per rhizosphere). Groups sharing the same letters are not significantly different. **B:** Comparison of carbon investment between AM (Dark green) and NM (Orange) plant communities represented as µg of freshly fixed carbon (^{13}C) incorporated into total microbial biomass per µg ^{15}N gained by plants with NM and AM plant community (n = 9 per community).

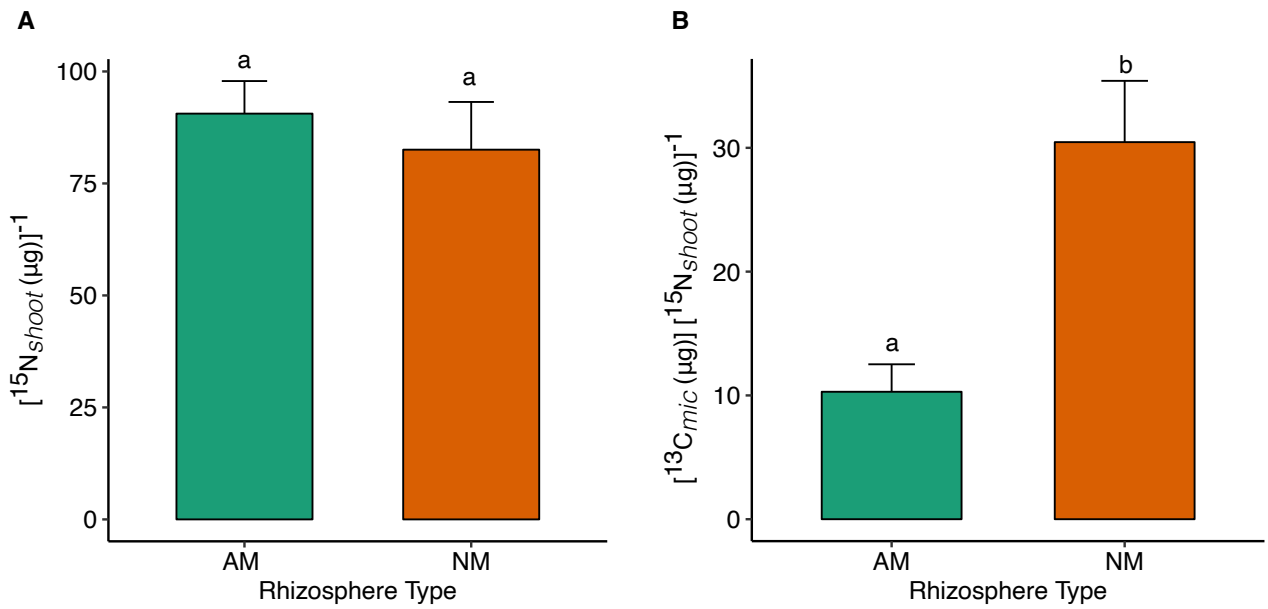
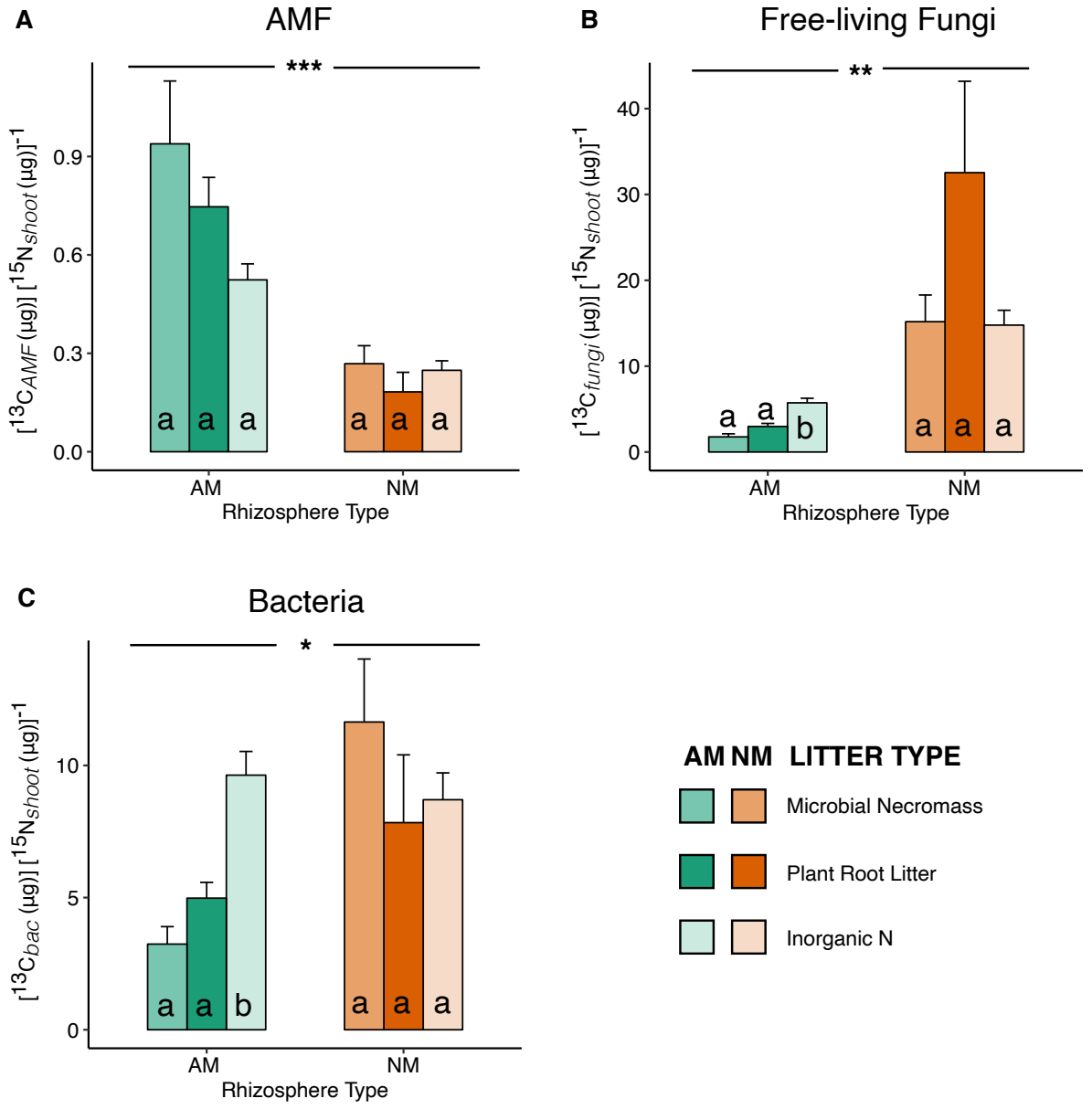


Figure 4: ^{13}C investment for ^{15}N gain by plants partitioned based on microbial groups

Partioning of plant ^{13}C investment into microbial biomass ($^{13}C_{mic}$) per ^{15}N gained by into key microbial groups like AMF (A), free-living fungi (B), and free-living bacteria (C) in rhizospheres of AM and NM plant communities amended with the three litter types (Plant Root Litter, Microbial Necromass and Inorganic N). Significant differences between the rhizosphere types are depicted with asterisks when $p < 0.05$ (*) , $p < 0.01$ (**) and $p<0.001$ (***) . Different letters within the bar is shows significant differences between litter types amended in that rhizosphere.

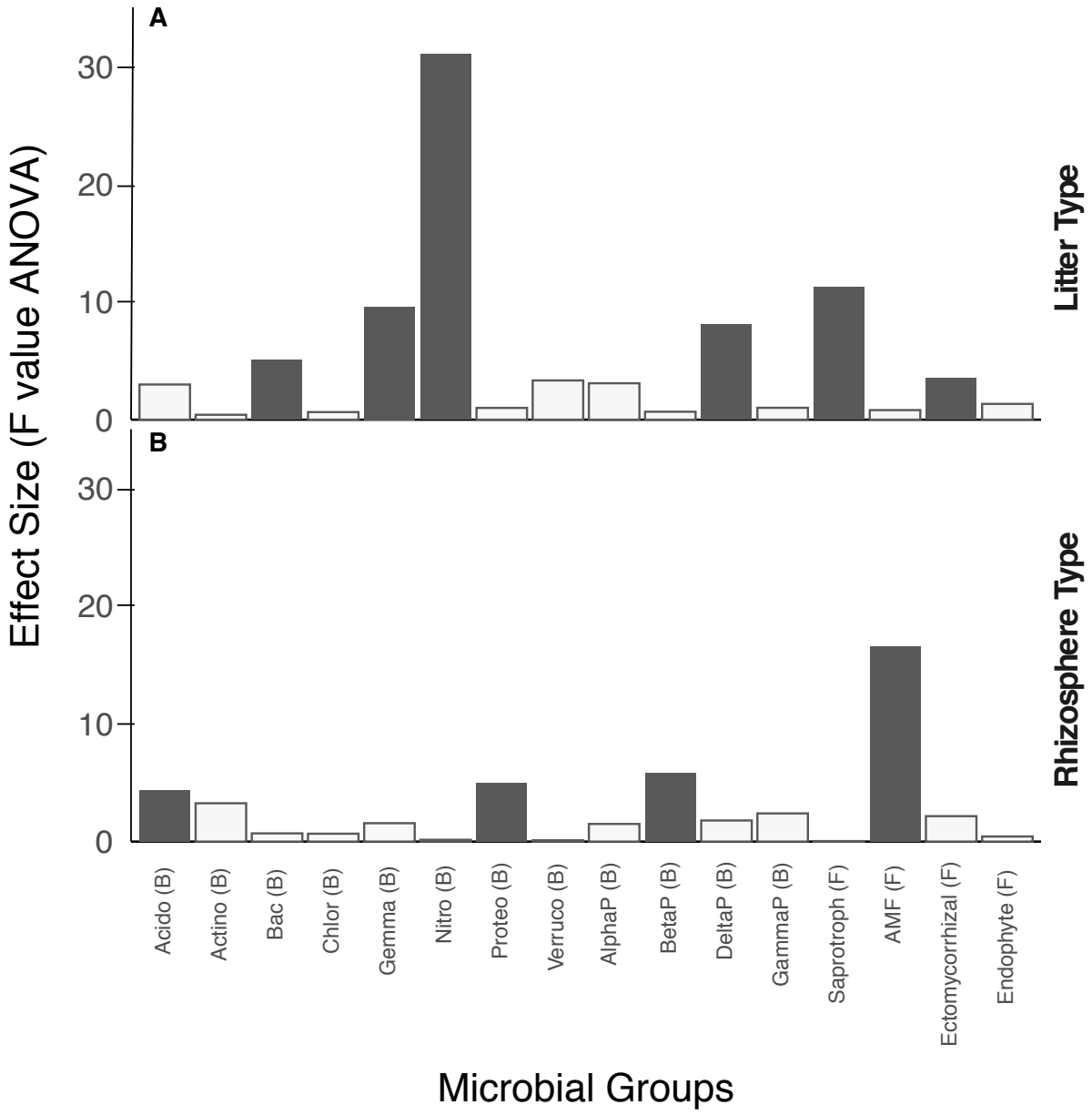


710

711 **Figure 5: Effects of rhizosphere type and litter type on specific microbial groups.**

712 Comparison of effect size (F values from two-way ANOVA) for relative abundance of
713 individual microbial groups in response to experimental treatments (Litter (A) and
714 Rhizopshere (B) types). Filled bars represent significant effect of a treatment on the relative
715 abundance of the microbial group. Relative abundances of bacterial and fungal groups can be
716 found in supplementary Figures S1, S2 and S3. Microbial group abbreviations – Nitro
717 (*Nitrospira*), Gemma (*Gemmamonadetes*), Actino (*Actinobacteria*), Chlor (*Chloroflexi*),
718 DeltaP (*Deltaproteobacteria*), BetaP (*Betaproteobacteria*), Proteo (*Proteobacteria*), AlphaP
719 (*Alphaproteobacteria*), GammaP (*Gammaproteobacteria*), Acido (*Acidobacteria*), Verruco
720 (*Verrucomicrobia*), Bac (*Bacteroidetes*).

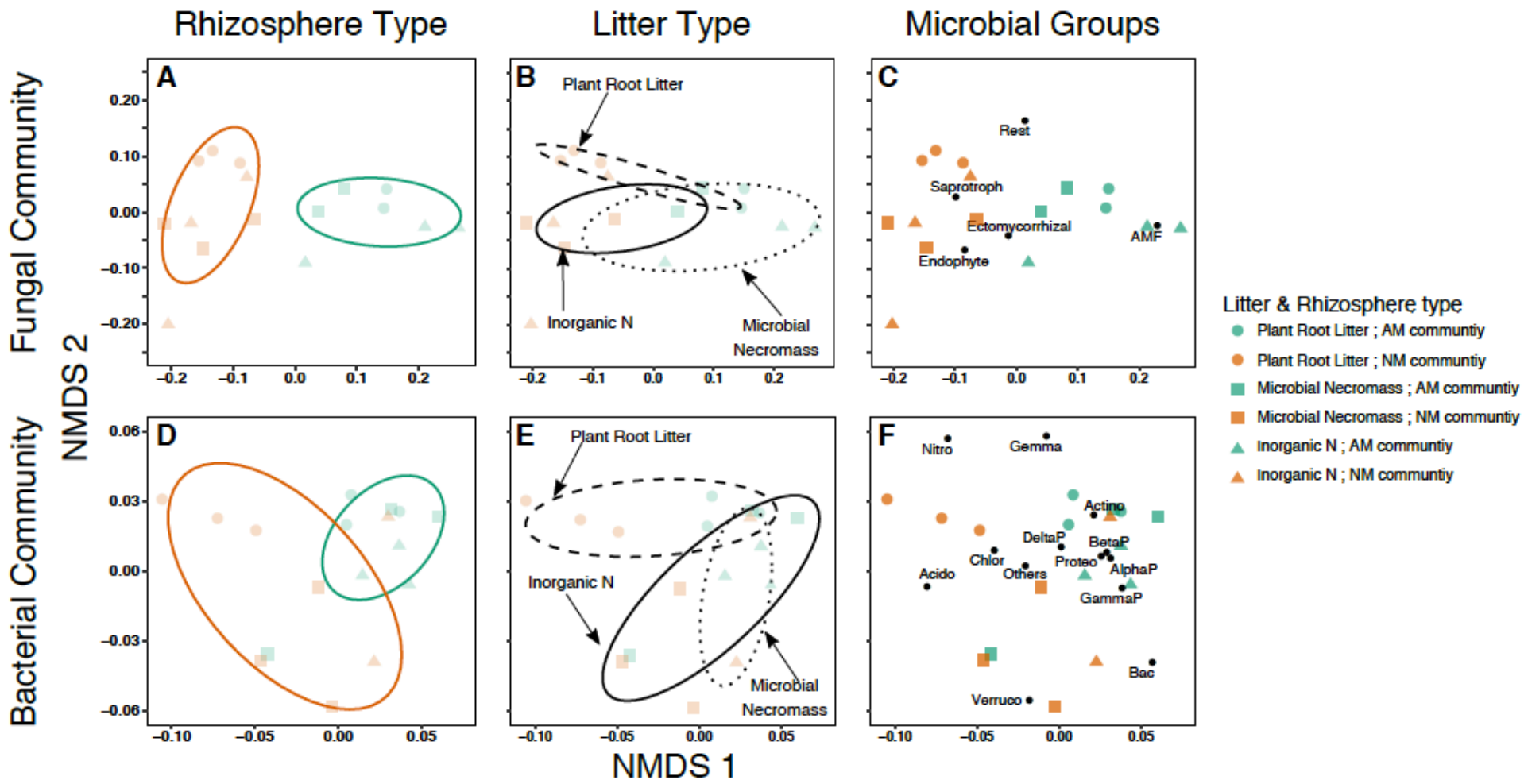
721


A
B
Effect Size (F value ANOVA)
30
20
10
0
Litter Type
Rhizosphere Type
Acido (B)
Actino (B)
Bac (B)
Chlor (B)
Gemma (B)
Nitro (B)
Proteo (B)
Verruco (B)
AlphaP (B)
BetaP (B)
DeltaP (B)
GammaP (B)
Saprotroph (F)
AMF (F)
Ectomycorrhizal (F)
Endophyte (F)
Microbial Groups

722

723 **Figure 6: Variation in microbial bacterial and fungal community composition**

724 Non-metric multidimensional scaling (NMDS) plots displaying Bray-Curtis distances
725 amongst microbial community composition under different combinations of rhizosphere types
726 (panel A&D) and litter types (panel B&E). Colors represent rhizosphere types where Dark
727 green= AM plant community and Orange= NM plant communtiy. Symbols represent litter
728 type being used with plant root litter (circle), Microbial necromass (square) and Inorganic N
729 (triangle). Microbial group abbreviations – Nitro (*Nitrospira*), Gemma (*Gemmamonadetes*),
730 Actino (*Actinobacteria*), Chlor (*Chloroflexi*), DeltaP (*Deltaproteobacteria*), BetaP
731 (*Betaproteobacteria*), Proteo (*Proteobacteria*), AlphaP (*Alphaproteobacteria*), GammaP
732 (*Gammaproteobacteria*), Acido (*Acidobacteria*), Verruco (*Verrucomicrobia*), Bac
733 (*Bacteroidetes*). Stress values for fungal (A, B &C) and bacterial (D, E &F) plots are 0.05 and
734 0.06 respectively.


Rhizosphere Type
Litter Type
Microbial Groups
Fungal Community
Bacterial Community
NMDS 2
NMDS 1
A
B
C
D
E
F
Plant Root Litter
Inorganic N
Microbial Necromass
Rest
Saprotroph
Ectomycorrhizal
AMF
Endophyte
Nitro
Gemma
Actino
DeltaP
BetaP
Chlor
Proteo
AlphaP
Acido
Others
GammaP
Bac
Verruco
Litter & Rhizosphere type
Plant Root Litter ; AM communtiy
Plant Root Litter ; NM communtiy
Microbial Necromass ; AM communtiy
Microbial Necromass ; NM communtiy
Inorganic N ; AM communtiy
Inorganic N ; NM communtiy

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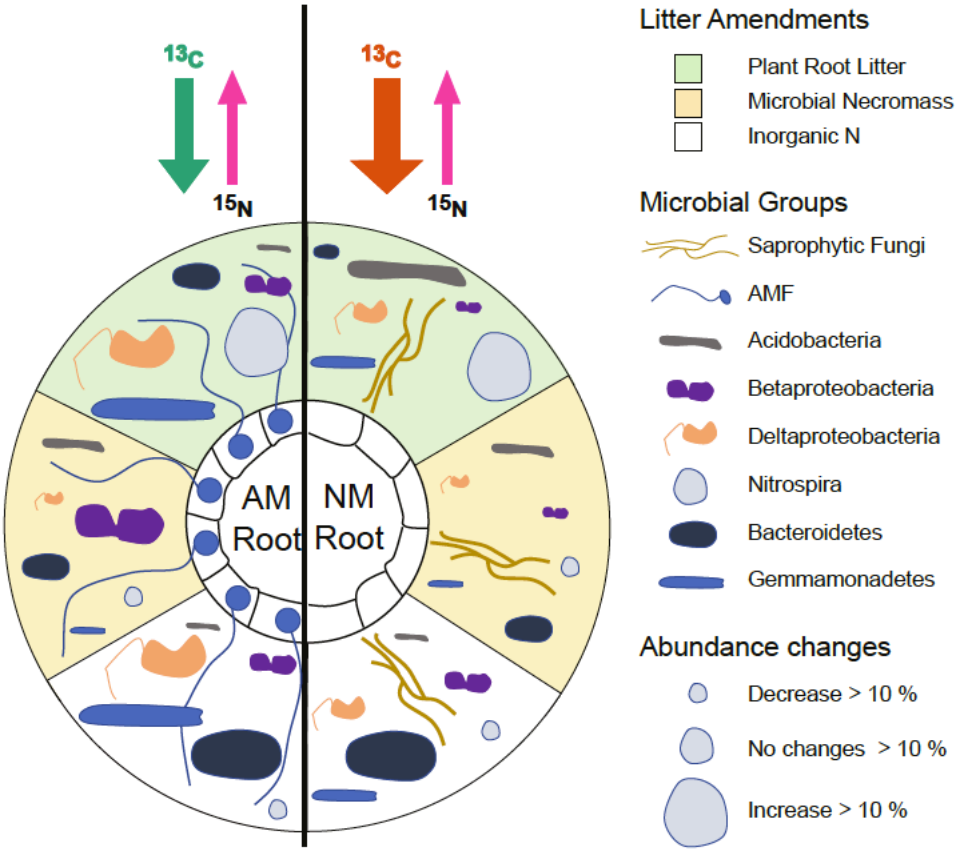
737

Figure 7 – Conceptual model showing key differences between plants associated with AMF and not associated with AMF. Arrows on each side represent the C and N flow differences arising due to presence or absence of AMF. The microbial community influenced by plant fresh C input and litter inputs are represented as sectors. Microbial groups that are specified in the key are responsive to either litter or plant inputs.



745 **Table 1: Permanova analysis of bacterial and fungal communities of the microcosms**

Microbial Community	Treatment	Df	F.value	R^2	p-value
Bacterial	**Rhizosphere Type**	**2**	**3.68**	**0.19**	**0.01**
	Litter type	**2**	**3.83**	**0.2**	**0.01**
Fungal	**Rhizosphere Type**	**2**	**16.22**	**0.57**	**0.00**
	Litter type	**2**	**3.40**	**0.12**	**0.05**

746

747 Rows in bold face represent significant effects. Interaction terms (Rhizosphere Type x Litter

748 type) were not significant.

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