Nutrient Source and Mycorrhizal Association jointly alters Soil Microbial Communities that shape Plant-Rhizosphere-Soil Carbon-Nutrient Flows.

Running title: Plant effects on nutrient cycling in the rhizosphere

Somak Chowdhury[1], Markus Lange[1], Ashish A Malik[2] Timothy Goodall[3], Jianbei Huang[1], Robert I Griffiths[4] and Gerd Gleixner[1]

Affiliations –

[1] Department of Biogeochemical Processes, Max Planck Institute for Biogeochemistry, 07745, Jena, Germany

[2] School of Biological Sciences, University of Aberdeen, Aberdeen, UK

[3] Centre for Ecology and Hydrology, Wallingford, Oxfordshire, UK

[4] Centre for Ecology and Hydrology, Bangor, UK

Corresponding author: Gerd Gleixner

Institutional Affiliation: Department of Biogeochemical Processes,

Max Planck Institute for Biogeochemistry,

07745, Jena, Germany

Email: gerd.gleixner@bgc-jena.mpg.de

Phone no: +493641576172

Competing Interests statement: The authors declare that they have no conflict of interest.

Keywords: AMF; Pulse labeling; ^{13}C; ^{15}N; ingrowth core; PLFA; 16S rRNA Gene; ITS;

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 uptake remains limited. Therefore, we performed a greenhouse experiment with two plant communities differing in their ability to associate with arbuscular mycorrhizal fungi (AMF). By applying a $^{13}CO_2$ pulse label to the plant communities and adding various ^{15}N labelled substrate 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 rhizosphere microbial communities and their community composition not only depended on plant-AMF association but also type of substrate being decomposed. AMF-association resulted in lower net ^{13}C investment into the decomposer community than absence of the association for similar ^{15}N uptake. This effect was driven by a reduced carbon flow to fungal and bacterial saprotrophs and a simultaneous increase of carbon flow to AMF. Additionally, in presence of AMF association CN flux also depended on the type of substrate being decomposed. Lower net ^{13}C assimilation was observed for decomposition of plant-derived and microorganism-derived substrates whereas opposite was true for inorganic nitrogen. Interestingly, the decomposer communities assembled in the rhizosphere were structured by both the plant community and substrate amendments which suggests existence of functional overlap between the two soil contexts. Moreover, we present preliminary

evidence that AMF association helps plants access nutrients that are locked in bacterial and plant necromass at a lower carbon cost. Therefore, we conclude that a better understanding of ecosystem processes like decomposition can only be achieved when the whole plant-microorganism-litter context is investigated.

Introduction

Plant growth is mostly limited by nutrient availability in soil, as nutrients in soil are either soil organic matter bound or associated with the mineral matrix (Veresoglou et al., 2012). Consequently, plants invest substantial parts of their photoassimilated carbon into soil via root exudates (sugars, amino acids and organic acids) and rhizodeposits, to stimulate microbial decomposition of soil organic matter and nutrient release (Jacoby et al., 2017; Kuzyakov and Xu, 2013). Additionally, root exudates can also alter organo-mineral associations (Keiluweit et al., 2015), pH (Malik et al., 2018) and redox potential (Husson, 2013) of the soil to directly improve nutrient availability. However, immediate plant uptake of available nutrients is not always possible (Dijkstra et al., 2013) since soil microbial communities have a high nutrient demand and often assimilate nutrients more efficiently than plants (Levin and Angert, 2015; Zhu et al., 2017). Since nutrient cycles in soil are largely controlled by the various biotic (root and microbial traits) and abiotic soil properties (minerology, pH and redox), variations in root exudation patterns and association between plants and microorganisms can have a tremendous impact on the rates of nutrient cycling in the rhizosphere (Craine et al., 2007; Veresoglou et al., 2012).

To enhance nutrient uptake from soil a majority of terrestrial plants species (> 80%) rely on direct root association with arbuscular mycorrhizal fungi (AMF) (Bonfante and Genre, 2010; Davison et al., 2015). Though AMF can't decompose organic matter themselves (Tisserant et al., 2013), they can use their hyphal network to scavenge nutrients and compete with the decomposer community for nutrient uptake (Averill et al., 2019; Bunn et al., 2019). The AMF network also benefits plants by allowing indirect root access to a greater soil volume (Sanders and Tinker, 1973), immobile nutrient pools (Hestrin et al., 2019) and distant water pockets (Bowles et al., 2018; Karlowsky et al., 2018), thus greatly increasing the area across which plant carbon is distributed. As a result, presence of AMF association ensures that fresh photoassimilates are allocated to the soil microbial community in a targeted manner

through the hyphal network (Kaiser et al., 2015) for nutrient exchange. The extent of this exchange however is regulated by the availability of nutrients in soil (Whiteside et al., 2019). In contrast, an absence of AMF association results in passive release of photoassimilated carbon across comparatively reduced soil volumes (Kaiser et al., 2015). To understand how these distinct modes of carbon delivery into soil affect nutrient cycles and plant microbial nutrient fluxes, it is essential to examine them in the context of various nutrient sources from which plants generally acquire nutrients.

Microbial decomposition processes are critical for sustained nutrient release in soil (Schimel and Bennett, 2004), however they are limited by the availability of labile carbon (Hütsch et al., 2002) - a primary source of which are root exudates. The same root exudates can also destabilize mineral nutrient associations and ensure nutrient release for the decomposer community (Jilling et al., 2018). As root-AMF associations can alter both the quantity (de Graaff et al., 2010) and biochemical profile (Shi et al., 2016; Zhalnina et al., 2018) of root exudates, they can have a large impact on nutrient availability in soil. Similarly, nutrient content and type of decomposing organic matter can significantly alter associated decomposer communities and their activity (Güsewell and Gessner, 2009; Meier et al., 2015; Strickland et al., 2009) to influence rates of nutrient cycling in soil. Recent findings based on functional analysis of rhizosphere microbial communities strongly indicate that a functionally diverse microbial community is maintained for exudate utilization in the rhizosphere compared to the bulk soil (Nuccio et al., 2020). Though exudate and litter associated shifts in the microbial community of the rhizosphere are well known, understanding the underlying carbon-nutrient flux between plant-AMF association and these decomposer communities across various nutrient sources can offer further insights into mechanisms of plant nutrient uptake.

Soil receives nitrogen containing substances either via carbon rich plant residues, nitrogen rich microbial residues or inorganic nitrogen from deposition and fertilizers (Gleixner, 2013;

108 Liang et al., 2017; Thuille et al., 2015). However, the difference amongst these inputs based
109 on nutrient content (litter quality) often hides the effect of their biochemical characteristics
110 (litter type) on decomposer community assembly and nutrient exchange. Under natural
111 conditions, the decomposer community is typically shaped by litter characteristics and their
112 physiological boundaries, defined mainly by their energetic and nutritional needs (Thomson
113 et al., 2013). As organic matter decomposition is mostly mediated by microbial communities,
114 a sizable portion of the soil carbon and nitrogen pool is of microbial origin (Xu et al., 2013).
115 Moreover, the porous nature of soil matrix and presence of complex surfaces ensures that
116 nutrient rich microbial residues are bound within nutrient poor exopolysaccharides as part of
117 soil biofilms (Kallenbach et al., 2016). Though exchange of nutrients between the microbial
118 pool and plants are commonplace, however, unlike plant root litter, little is known about how
119 their turnover impacts carbon-nutrient flux during litter decomposition in the rhizosphere
120 (Nuccio et al., 2020), thus underscoring the need for further investigation through labelled
121 nutrient amendments.

122 In summary, large uncertainties still exist regarding the mechanistic benefits of plant-
123 AMF associations with respect to organic matter source and nutrient return. Therefore, we
124 assembled two plant communities that differed in their ability to associate with AMF (AM or
125 NM) and amended the root colonized soil with three ^{15}N labeled nitrogen substrates (plant
126 root litter, microbial necromass and inorganic N) of similar quality (C:N ratio) and traced the
127 net plant derived carbon flow into the microbial community (PLFA/NLFA) using a $^{13}CO_2$
128 pulse label experiment. We also investigated the microbial community composition using
129 DNA and RNA based amplicon sequencing. We first hypothesized that AMF associations
130 result in greater plant carbon export to the microbial community (including AMF) for
131 nitrogen mineralization. We also hypothesized that carbon cost per nutrient gain is higher in
132 presence of AMF association than absence, since AMF associations require considerable
133 amounts of plant carbon for maintaining the hyphal network. We finally hypothesize that the

associated soil microbial community depends in the first order on the plant community (AMF vs Non-AMF) and its carbon export, and in the second order on the substrate origin independent from the total amount of added nutrients.

Materials and Methods

Microcosm Experiment

The soil used for the experiment was collected from the Jena Experiment - a grassland biodiversity experiment located in Jena, Germany in March 2015. The soil type was Eutric Fluvisol (Roscher et al., 2004) which has a pH of 7.4 (s.d. 0.06), organic carbon content of 21.1 g C kg^{-1} (s.d. 3.6 g C kg^{-1}) and total nitrogen content of 2.2 g C kg^{-1} (s.d. 0.3 g N kg^{-1}) (Weisser et al., 2017). The soil contains a diverse mixture of bacteria and fungi (Weisser et al., 2017) that served as a natural inoculum in our experiment. After removing the root mat in the upper 2 cm, the top 5 cm of soil was collected, sieved (<2 mm) and all remaining roots were removed. The soil was well mixed and stored at 4 ^{o}C until the establishment of the pots 1 week later. We set up microcosms (19 x 12.5 x 9.5 cm) and established mixed temperate plant communities of two types namely AMF dependent plant community (*Linum ussitatissimum, Linum perenne, Festuca rubra* and *Tagetes patula*) termed as the AM plant community and plant community lacking in AMF associations (*Dianthus caryophyllus*, *Matthiola longipetala sub-species bicornis*, *Dianthus barbaratus* and *Carex arenaria*) termed as NM plant community (Brundrett, 2015b, 2015a). By using plant communities instead of monocultures, we intended to avoid well-known species-specific effects of root exudation. Therefore we used 4 species mixtures that have already been shown to overcome species specific effects (Weisser et al., 2017). Therefore, our experiment provides plant community level understanding of nutrient flux between plants and microorganisms.

Each microcosm had a single central PVC ingrowth core (diameter = 5 cm) with two tiers of 8 windows (4 x 1.5 cm) guarded by a 1000 micron mesh (Figure S1). No external

159 inoculum of AMF was used as the source soil already contained a diverse AMF community as
160 confirmed by types of plants prevalent at the site (Harley and Harley, 1987). For both plant
161 communities (AM and NM) we used a 1000 micron mesh to ensure the growth of fine roots
162 and hyphae into the ingrowth core (Figure S1). We confirmed root access at the time of
163 substrate amendment and final harvest (Figure S2). The following replication scheme was
164 implemented in the experiment 3 biological replicates x 3 soil amendments x 4 timepoints
165 resulting in a total of 36 independent microcosms per plant community. The microcosms and
166 the ingrowth cores were filled with well mixed soil at field bulk density and were pre-
167 equilibrated for 2 weeks in the greenhouse. Even plant communities for each plant community
168 type were established by planting 1 seedling per species that already developed first true
169 leaves within each microcosm unit outside the ingrowth core resulting in a final plant species
170 richness of 4 in each microcosm (Figure 1). The microcosms were randomly distributed in the
171 greenhouse and maintained for 4 months until the plants were fully developed. Irrigation was
172 provided using an ebb and flood system for 5 min daily and lighting for the plants (Son- T
173 Agro 430 W HPS bulbs, primary light range = 520–610 nm, Philips Lighting Company,
174 Somerset, NJ, USA) was provided daily for 12 h. Plant species richness and evenness were
175 visually monitored throughout the experiment until the time of harvest.

176 In order to make substrate amendments to fully developed microcosms, the central
177 ingrowth core was removed. The soil was sieved (<2mm) to remove fine roots (Refer Section
178 1.2 of Supplementary Information). The N amendments made to ingrowth core soil differed in
179 their origin (plant, microbial and inorganic). The plant root litter and microbial necromass
180 differed in their native C:N ratio with plant root litter having a C:N ratio of 45.67 $\pm$ 1.09 (C
181 content = 7.41 % and N content = 0.16 %) and microbial necromass had a C:N ratio of 8.37 $\pm$
182 1.35 (C content = 12.88 % ; N content = 1.54 %). For plant root litter amendment, sieved
183 ingrowth core soil was uniformly mixed with 7.5 g of shredded plant root litter which resulted
184 in an input of 48.55 μg of ^{15}N into the soil. For microbial necromass amendments, sieved soil

185 was amended with 0.8 g of microbial necromass containing 49.53 μg of ^{15}N with an
186 additional 1.28 g of nitrogen free xanthan resulting in an additional carbon amendment of
187 0.448 g which ensured a similar input of carbon and nitrogen as that of plant root litter. The
188 inorganic-N amendment with KNO_3 was carried out by mixing 0.012 g of KNO_3 (^{15}N content
189 = 48.25 μg) with 1.58 g of xanthan resulting in a total C input of 0.553 g C, thus ensuring a
190 similar carbon and nitrogen input as that of plant root litter amendment. Overall, the
191 amendments were kept small, the total carbon and nitrogen content of the ingrowth core soil
192 increased by ca. 0.15 % and ca. 0.01 %, respectively. The amended soil was mixed, refilled
193 into the ingrowth core and reinstalled on the microcosm.

194 2 weeks after the amendment, which allowed roots and hyphae to regrow into the
195 ingrowth core, the microcosms were exposed to a $^{13}CO_2$ pulse in an air tight plexiglass
196 chamber (Malik et al., 2015). For each litter amendment, all microcosms (3 replicates x 2
197 plant treatments x 3 post-labelling timepoints = 18 microcosms) were subjected to a $^{13}CO_2$
198 pulse together in a well-lit (PAR intensity >500 μmol photons $m^{-2}s^{-1}$) chamber (volume =
199 0.15 m^3). The chamber air was flushed with CO_2 free synthetic air until the CO_2 concentration
200 fell below 150 ppm. Enriched $^{13}CO_2$ (99 atom %) was introduced into the chamber using a
201 mass flow controller (model- MF1-20sccm-N2, MKS Instruments Inc.) at a flow rate of 3.33
202 x 10^{-7} m^3s^{-1} for 10 min and circulated using internal fans. The CO_2 concentration was
203 monitored online by a cavity ringdown spectrometer (G2101-I, Picarro Inc., Santa Clara, CA,
204 USA) throughout the labeling period of 1h. An additional maintenance stream of $^{13}CO_2$ was
205 introduced halfway at a flow rate of 1.67 x 10^{-7} m^3s^{-1} for 5 min that kept the chamber CO_2
206 concentration between 350 ppm and 410 ppm. The chamber temperature was maintained
207 close to greenhouse temperature by placing racks of frozen ice packs along the vertical walls
208 of the chamber and replaced for every batch of pots that were labelled.

209 At the end of the labelling period the microcosms were removed from the chamber
210 and returned back to the greenhouse and destructively harvested at 4 h, 24 h and 240 h from

211 the pulse. The soil from ingrowth core was completely inhabited by roots (Figure S2)
212 suggesting that all soil in the ingrowth core was rhizosphere soil. The roots were removed by
213 sieving to 2mm sieve and hand picking (SI Section 1.2) and remaining rhizosphere soil was
214 divided into three sub-samples and stored at -80 ^{o}C for molecular analysis within 1 h of
215 harvest, dried at 105 ^{o}C for 48 h and stored at room temperature for elemental analysis and at
216 -20 ^{o}C for microbial biomass and respiration analysis. The above and below ground plant
217 tissues were collected at the last destructive harvest (240 h) and dried at 60 ^{o}C for 48 h and
218 subsequently stored for future analysis. Prior to $^{13}CO_2$ labelling of microcosms, control pots
219 for each litter amendment (3 replicates x 3 soil amendments x 2 plant community treatments =
220 18 microcosms) were also placed for an hour in the chambers with similar synthetic CO_2 free
221 air and $^{12}CO_2$ pulsing regimes as the labelled samples with similar temperature maintenance
222 and chamber ventilation. To avoid cross contamination from ^{13}C labeled air, the control
223 microcosms were harvested prior to labelled pots.
224

225 **Production of labelled substrates**

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

235 Inoculum for producing microbial necromass was generated by slow centrifugation of
236 6 g of Jena Experiment soil in 20 ml of distilled water at 650 g for 15 min. The supernatant

237 was inoculated into M9 minimal medium amended with 20% glucose and contained $K^{15}NO_3$
238 (0.40 atom%) as only nitrogen source. The culture grew for 7 days at 19 °C on a horizontal
239 shaker. The entire cell mass was harvested by centrifugation (@ 3500 g for 15 min) and re-
240 suspended in 10 ml of sterile distilled water and sterilized by autoclaving. The sterile pellet
241 was freeze dried for further use.

242 The inorganic nitrogen substrate was generated by mixing KNO_3 and $K^{15}NO_3$ such
243 that the mixture had an isotopic signature of 0.40 atom % and used directly for the
244 amendments with above mentioned C:N ratio adjustment.

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

246 The shoot and root samples (one mixed sample per pot) that were harvested at the end
247 of the experiment (240h sample for both plant communities) were subjected to carbon and
248 nitrogen elemental and isotope analysis (3 replicates x 3 amendments x 2 plant treatments =
249 18 samples). Briefly, oven dried shoot and root biomass was ball-milled to a fine powder.
250 Single tin capsules were prepared for total C and N content analysis and analyzed with vario
251 EL CNS analyzer (Elementar Analysensysteme GmBH). For bulk ^{13}C and bulk ^{15}N analysis
252 finely powdered shoot and root samples were weighed into separate tin capsules. They were
253 analyzed using an elemental analyzer EA1100 (CE, Mainland) coupled via a conflow III
254 interface to a DELTA+ (Finnigan MAT) isotope ratio mass spectrometer (Malik et al., 2015).

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

256 To assess the ^{13}C flow into the microbial community, fatty acids from neutral lipids
257 (NL) and phospholipids (PL) were extracted from ingrowth core soil sampled at 0 h and 240
258 h. All biological replicates of each plant community – soil amendment combination that were
259 collected at the same time point were pooled and homogenized to create a composite sample
260 from which 5g aliquots of soil were used for lipid extraction (1 composite sample x 2 plant
261 community x 3 soil amendments = 6 samples per time point). We applied a modified Bligh
262 and Dyer (1959) lipid extraction protocol using pressurized solvent extraction, as described

263 earlier in Karlowsky et al., 2018a. The concentration of ^{13}C incorporated relative to an
264 unlabeled sample (0h) for bacterial PLFA markers (14:0, 15:0, 16:0, 17:0, 18:0, 14:0i, 15:0i,
265 15:0a, 16:0i, 17:0i, 17:0a, 16:0(10Me), 17:0(10Me), 18:0(10Me), 17:0cy, 19:0cy, 15:1,
266 16:1ω7, 16:1ω5, 16:1, 17:1, 18:1ω7 and 18:1ω9) and fungal PLFA (18:2ω6,9) was
267 determined using a gas chromatograph coupled with a flame ionization detector (FID) and
268 isotope ratio mass spectrometer (IRMS) respectively (Karlowsky et al., 2018). Additionally,
269 the NLFA biomarker 16:1ω5 (a storage lipid) was used as the marker for AMF.

270 The amount of ^{13}C incorporated was determined from samples collected after the pulse
271 minus the isotope content of unlabeled soil collected before the pulse (Karlowsky et al.,
272 2018). The amounts of saprotrophic fungal markers (PLFA - 18:2ω6,9) were converted into
273 biomass C values by applying conversion factor 11.8 nmol = 1 mg C (Klamer, 2004), for
274 bacterial biomarkers by applying conversion factor 363.3 nmol = 1mg C (Frostegård and
275 Bååth, 1996) and for 16:1ω5 NLFA by applying conversion factor 1.047 nmol = 1μg C
276 (Williams et al., 2017). The amount of ^{13}C in the specific biomarker was determined from the
277 C pool size of the biomarker corrected for soil weight and moisture (Karlowsky et al., 2018).
278 The total amount of ^{13}C in the bacterial and fungal biomarkers was used as a proxy for
279 microbial biomass that incorporated freshly fixed plant C. Net ^{13}C assimilated by soil
280 microbial community integrated across total weight of ingrowth core soil and its parts
281 (saprophytic fungi, AMF and free-living bacteria) for every unit of ^{15}N gained by the plant
282 (n=3 per soil amendment) integrated across total above ground biomass was used as an
283 estimate of net plant C allocation for nutrient gain to specific microbial groups.

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

285 To ascertain the microbial community composition, 1.09 ± 0.01 g (3 replicates x 2
286 plant treatments x 3 amendments x 1 timepoint (0 hr) = 18 samples) of soil obtained from
287 control samples was used to co-extract total RNA and DNA using the MoBio RNA Powersoil
288 kit. The samples were bead-beaten for one cycle at 5.5 m/s for 30 s with a FastPrep

289 instrument (MP Biomedicals Germany GmbH). DNA was extracted using a MoBio RNA
290 Powersoil DNA elution accessory kit. Traces of DNA that was co-extracted in the final RNA
291 elution were removed using a MoBio DNase Max kit. The purified DNA and RNA
292 concentrations were measured using Qubit 4 fluorometer (Thermo Fisher Scientific) using the
293 broad range assay kits for DNA and RNA. Additionally, the purity of the samples was
294 checked using a nanodrop spectrometer 2000c (Thermo Fisher Scientific) and all samples
295 were found to have A260/280 and A260/230 ratios of 2 suggesting no protein or organic
296 compound contamination. The integrity of the RNA samples was visually verified using 0.8
297 % agarose gel electrophoresis. Presence of sharp well-defined ribosomal RNA bands and
298 smear in between and above these bands was considered as an indicator of high integrity of
299 the RNA. A replicate was dropped for AM plant community amended with plant root litter
300 and microbial necromass due to poor RNA integrity and subsequent non-amplification of
301 cDNA. 200 ng of total RNA extracted was converted into cDNA using Superscript^{TM} VILO
302 kit (Thermo Fisher Scientific) using random hexamers to ensure uniform conversion of all the
303 RNA fragments. The DNA and cDNA samples were further used for amplifying the 16S
304 rRNA gene and ITS regions respectively. Briefly, for the 16S rRNA gene sequencing,
305 amplicon libraries were prepared with V3-V5 primers (Haas et al., 2011) using PCR
306 conditions described by Kozich et al., 2013. One 16S rRNA gene amplicon replicate
307 associated with soil amended with Inorganic-N under NM plant community was dropped due
308 to non-amplification of extracted DNA. ITS2 gene libraries were constructed by amplifying
309 ITS region 2 using the fITS7 (forward) and ITS4 (reverse) primers (Ihrmark et al., 2012) with
310 previously described PCR conditions (Gweon et al., 2015). Both sets of primers also
311 consisted of an appropriate Illumina adapter, an 8-nt index sequence, a 10-nt pad sequence, a
312 2-nt linker (Kozich et al., 2013). The amplicons were sequenced using Illumina MiSeq, v3
313 chemistry, 600 cycles.

314 **Processing of sequencing data**

315 For the bacterial community 16S rRNA gene sequencing data, the forward and reverse
316 reads obtained were merged using PEAR (v 0.9.6) with default parameters (Zhang et al.,
317 2014). The bacterial community composition was analyzed using the QIIME (1.8.0) pipeline
318 (Caporaso et al., 2010). Representative OTU's were picked using UCLUST using de novo
319 method at 97% similarity level and were assigned taxonomy using RDP classifier method
320 with the SILVA reference data set (release 132 for QIIME) (Edgar, 2010; Wang et al., 2007).
321 Total number of sequencing reads classified within the OTU table for the samples varied
322 between 4000 to 21982 reads. In order to make inter-sample comparisons OTU table obtained
323 was rarefied to 4000 sequences per sample using QIIME command single_rarefraction.py
324 (Caporaso et al., 2010).

325 Since fungal spores are ubiquitous, the ITS2 regions that were transcribed into RNA
326 were used to sample transcriptionally active fungi (Anderson and Parkin, 2007). The
327 amplified ITS region from total cDNA samples were used for sequencing. The amplified ITS
328 sequencing data was analyzed using the PIPITS pipeline using the default parameters (Gweon
329 et al., 2015). Briefly, the paired end sequences were merged using VSEARCH after which the
330 ITS2 region was extracted using ITSx after which they are matched to a trained UNITE
331 database at 97% similarity using RDP classifier to obtain an OTU table (Bengtsson-Palme et
332 al., 2013; Rognes et al., 2016). The OTUs detected were then assigned to fungal guilds using
333 FUNguild assignment app (http://www.stbates.org/guilds/app.php) (Nguyen et al., 2016). The
334 proportion of ITS community assigned to AMF was also used as evidence of AMF
335 regrowth/presence in the ingrowth core.

336 The bacterial and fungal amplicon sequencing data and the associated metadata is
337 publicly available through the Sequence Read Archive project number: SRP132171. A
338 detailed table of associated metadata and download links is also provided in the
339 supplementary information (Table S12 and S13).

340 **Statistical data analysis**

341 One-way ANOVA was used to determine statistical differences amongst rhizosphere
342 types (AM vs NM) for root:shoot ratio, ^{15}N assimilated by plant shoot and net microbial ^{13}C
343 assimilation into total microbial biomass. Two-way ANOVA was applied to identify
344 statistically significant differences due to rhizosphere and litter types for plant carbon
345 allocation per unit ^{15}N gain. All two-way ANOVA models that showed significant
346 interactions of rhizosphere-litter types were subject to Tukey's post-hoc test to identify
347 differences amongst litter types nested within rhizosphere types. Significant differences were
348 only considered if the assumption for homogeneity of variance was met using the Flinger-
349 Killing test (Table S2). Subsampled OTU tables were binned to different taxonomic groups at
350 the phylum and class levels (bacteria) whereas fungal OTUs were binned into guilds to
351 compare effect of AM vs NM plant communities and substrate amendments on microbial
352 (bacterial and fungal) community composition. The effects of substrate and plant community
353 on microbial groups was determined using a two-way ANOVA of their relative abundance.
354 Differences between microbial (bacterial and fungal) community composition affected by the
355 experimental treatment were determined using non-parametric Permutational Multivariate
356 Analysis of Variance PERMANOVA ("adonis" function in the vegan package 2.5-2)
357 (Oksanen et al., 2018). The assumption of homogeneity of dispersions (variance) was tested
358 using the betadisper function (vegan package 2.5-2) and both the fungal and bacterial
359 community composition was found to meet this assumption (Table S9). The dissimilarity
360 based upon microbial community composition due to litter and rhizosphere treatments were
361 visualized as an NMDS ordination of Bray-Curtis dissimilarity coefficients was plotted to
362 visualize the dissimilarities between the microbial communities under the influence of plant
363 communities and substrate added to the soil. All analysis was performed using R software (v
364 3.4.3) with visualization being done with ggplot2 package (Wickham, 2011).

Results

Carbon allocation and Nitrogen return

The AM plant community had a lower shoot biomass (4.62 ± 0.14 g, n = 9 aggregated across substrate amendments) compared to NM the plant community (7.42 ± 0.44 g, n = 9 aggregated across substrate amendments) but both had similar root biomass ($F_{1,16}$ = 0.17; p = 0.685), which resulted in a higher root to shoot ratio for the AM plant community. The AM plant community also allocated more carbon into their roots, which was reflected in the root to shoot ratio of ^{13}C incorporated into the AM plant community compared to the NM plant community ($F_{1,16}$ = 20.91, p < 0.001; Figure 2A). Unexpectedly, the net ^{13}C assimilation of the rhizosphere microbial communities from the AM plant community was significantly lower than that of the NM plant community ($F_{1,16}$ = 21.75, p < 0.001, Figure 2B).

The AM plant community gained 93.1 ± 4.7 μg ^{15}N from litter decomposition, which was not significantly different from the NM plant community that gained 82.3 ± 10.7 μg ^{15}N (($F_{1,16}$ = 0.40, p = 0.541, Figure 3A). A comparison across substrate amendments nested within either plant community showed no significant differences in terms of ^{15}N uptake (AM: $F_{2,6}$ = 0.57, p = 0.594; NM: $F_{2,6}$ = 0.797, p = 0.493). However, the AM plant community invested 66% less ^{13}C into the microbial community per ^{15}N gain than the NM plant community ($F_{1,16}$ = 15.20, p = 0.001; Figure 3B). Moreover, the AM plant community allocated significantly more ^{13}C per ^{15}N gained to AMF biomass ($F_{1,16}$ = 30.53, p < 0.001; Figure 4A) whereas NM plant communities allocated significantly more ^{13}C per ^{15}N gained into saprophytic fungi ($F_{1,16}$ = 15.47, p = 0.001; Figure 4B) and bacteria ($F_{1,16}$ =4.78, p=0.044). Unexpectedly the amount of ^{13}C allocated per ^{15}N gained depended only in the AM plant community on the type of decomposed substrate ($F_{2,15}$ = 7.13, p = 0.009, Figure 4). Specifically, in the AM rhizosphere, colonization of inorganic N amended soil resulted in significantly more plant-derived ^{13}C to be allocated to net bacteria and saprotrophic fungal

390 biomass compared to microbial necromass and plant litter amended soil (Figure 4B, C). Such
391 litter-based differences were missing in the NM rhizosphere.
392

393 **Microbial community composition**

394 The microbial communities fostered by presence or absence of AMF associations
395 were similar in terms of richness and evenness (Table S3 and S4). However, individual
396 bacterial groups and fungal guilds were responsive to either presence or absence of AMF
397 association (AM and NM) or to specific substrate amendments (plant root litter, microbial
398 necromass and inorganic-N). Presence of AMF association significantly increased relative
399 abundance of bacterial groups belonging to *Actinobacteria* and *Proteobacteria* (specifically
400 *Betaproteobacteria)* (Table S5) whereas absence of AMF associations significantly increased
401 the abundance of the *Acidobacteria* group (Table S5). In agreement with results from PLFA
402 analysis, absence of AMF association also significantly increased reliance of the plant
403 community on active fungal saprotrophs and endophytes (Table S6), both of which are guilds
404 that can lead a free-living lifestyles (Rodriguez et al., 2009). In contrast and as expected, the
405 presence of AMF associations resulted in significantly higher relative abundance of
406 symbiotrophic AMF guilds (Table S6) and reduced abundance of free-living fungal guilds
407 (Saprotrophs and Endophytes). As the fungal ITS community was determined using cDNA-
408 based amplicon sequencing, dominance of AMF along with copious root ingrowth (Figure
409 S2) was also used as confirmatory evidence of AMF colonization of ingrowth core soil.

410 Substrate amendments on the other hand elicited specific responses in different
411 bacterial groups depending on presence or absence of AMF associations. Bacterial groups
412 belonging to *Gemmamonadetes*, *Nitrospira* and *Deltaproteobacteria* were observed to be
413 significantly affected by substrate amendments (Table S7). In all cases, the differences could
414 be consistently explained by substrate source, with higher abundances of these groups
415 observed in ingrowth cores amended with plant root litter relative to other amendments. The

416 fungal OTUs that were not exclusively assigned to major guilds (Saprotrophs, AMF,
417 Ectomycorrhizal and Endophytes) were observed to show a significant response to substrate
418 amendments (Table S8).

419 Fungal community composition varied on expected lines with presence or absence of
420 AMF association explaining dissimilarity amongst microbial communities (Figure 5A).
421 Ordinations of fungal communities also revealed that effects of substrate amendments were
422 nested within plant community treatment (Figure 5A). Ingrowth core fungal community
423 composition in presence of AMF association depended mainly on type of nitrogen source
424 with clear separation of communities between organic and inorganic nitrogen sources (Figure
425 5A). On the other hand, ingrowth core microbial communities in absence of AMF association
426 were separated by substrate origin with a clear separation between plant root litter and
427 remaining amendments (Figure 5A). Bacterial community compositions were largely
428 dependent on substrate source (Figure 5B). Within these substrate amendments AMF
429 association resulted in sub clusters for plant root litter and inorganic N amendments only
430 (Figure 5B).

431 Permutational Multivariate Analysis of variance (PERMANOVA) revealed that both
432 fungal (Table S10) and bacterial community (Table S11) compositions were significantly
433 affected by plant community and substrate amendments. In terms of variance explained by
434 presence or absence of AMF association, fungal community composition was affected more
435 than bacterial community. In both bacterial and fungal community substrate amendments
436 explained a greater proportion of the variance in community composition.

437 Discussion

438 In this study we investigated how plant communities, differing in their association
439 with AMF, affected the carbon allocation to the microbial communities and the return of
440 mineralized nitrogen from different substrates. By combining a dual stable isotope labelling

441 with a sequencing analyses and compound specific isotope analysis of the soil microbial
442 community we found that AMF associated (AM) plant communities did not gain significantly
443 more nitrogen from the different substrates than non-mycorrhizal (NM) plant communities.
444 However, AM plant communities invested less carbon into the soil microbial communities
445 than NM communities for similar nutrient uptake (Figure 6), which was confirmed by total
446 microbial biomass (chloroform fumigation) and soil respiration (Figure S4 and S5). Even
447 though AM plant communities foster a carbon demanding fungal network, they benefit
448 strongly from the AMF's targeted access to the decomposition sites where plant-derived
449 carbon is used to stimulate selected microbial groups for substrate decomposition. This
450 important role of the hyphae is underscored by the fact that a comparison of compartments
451 with only hyphae access (30µm mesh) and root and hyphae access (100 µm mesh) did not
452 differ in their performance (Table S1). These findings are also in line with previous studies
453 showing the importance of the AMF hyphal network on the associated soil bacterial
454 community (Drigo et al., 2010; Vestergård et al., 2008). The close interaction of AMF and
455 bacteria potentially increases decomposition efficiency and makes in the long run more
456 nutrients available to the plant communities. NM plant communities likely miss the targeted
457 growth of AMF hyphae and rely on free-living saprophytic fungi and bacteria for
458 decomposition, therefore increasing the overall carbon per nitrogen cost.

459 Furthermore, our results also showed that the net assimilation of plant carbon by the
460 rhizosphere microbial community depended on the biochemical characteristics of the
461 substrates available for decomposition. This strongly suggests that substrate (bio)chemistry is
462 as important as nutrient content of the substrate in the decomposition process and can
463 modulate the extent of plant-derived carbon required for decomposition (Jones et al., 2004).
464 AMF-associated rhizosphere microbial communities generally assimilated less plant carbon
465 than the microbial communities lacking this association, which suggests that AMF allocated
466 less labile carbon to the free-living soil microbial community in order to take up mineralized

467 N from decomposition processes in soil. The different net assimilation of the free-living
468 microbial community from AM and NM plant communities also suggests that the
469 decomposition of different substrates strongly depended on the association with AMF. This
470 confirms that AMF indeed act as target-oriented root extensions that more effectively feed
471 plant-derived carbon to the free-living microbial community. Moreover, AMF through their
472 response to substrate biochemistry and nutrient availability likely control the amount of
473 exudates released for supporting decomposition related processes. In turn, the significant
474 response of specific microbial groups to either the litter type and rhizosphere type as well as
475 the shift in the microbial community composition suggests that in addition to the substrate
476 nutrient content (Stempfhuber et al., 2016) the interplay of substrate source with plant carbon
477 input also shapes microbial communities in the rhizosphere. Hence the composition of the
478 microbial decomposer community is adjusted to plant exudation and type of the substrate
479 being decomposed (Figure 6), making AMF associations with plants an important part of the
480 decomposition process (McLaren and Turkington, 2011). Expectedly, the fungal community
481 primarily differed between AM or NM plant communities, emphasizing the fungal role in
482 decomposition and nutrient gathering (Bukovská et al., 2018; Meier et al., 2015). However, in
483 the presence of AMF, we observed lower plant carbon assimilation by the free living
484 microbial community (Mueller et al., 2014), indicating dominance of bacterial community in
485 the associated decomposition processes. This suggests that primary decomposers in the AM
486 rhizosphere were bacteria that were fed and adapted to substrate types rapidly by the AMF
487 (Herman et al., 2012; Nuccio et al., 2013). Most interestingly our results indicate for the first
488 time that AM plant communities strongly benefit from the association with AMF during
489 nutrient uptake from microbial necromass. AMF assimilated more ^{13}C from plants but
490 invested less carbon into the bacterial community for decomposition. This might indicate that
491 AMF, which have no capability to decompose lignocellulose structures of plant cell walls
492 themselves (Tisserant et al., 2013), might be able to decompose peptidoglycan structures of

493 microbial cell walls. This idea would be supported by the dramatic up-regulation of genes
494 encoding for enzymes containing a peptidoglycan binding LysM domain and glycan
495 hydrolases like lysozyme, glucosamidase and alpha amylase that would be necessary for
496 microbial cell wall decomposition (Tisserant et al., 2013; Gude et al., 2012). If we consider
497 that large parts of soil organic matter are microbial necromass (Gleixner, 2013) the
498 association of plants with AMF gain a new overall importance for ecosystem functioning.
499 However, additional experiments are needed to confirm this new role of plant-AMF
500 associations for microbial cell wall decomposition.
501

Conclusion

Our results suggest that a three-way interaction between the plant community, the microbial community and the type of decomposed substrate is of fundamental importance for ecosystem functioning. Specifically, nutrient recycling in the plant-microorganism-soil continuum is shaped by these interactions leading to a plant-driven and context-dependent stimulation of decomposers whose decomposition capabilities are likely versatile as stoichiometrically identical litter differing in its origin are decomposed by different parts of the microbial community. Consequently, organic matter 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 AMF association is less carbon intensive and likely more targeted than passive exudation of plant communities lacking an AMF association. This might explain why a large part of the plant community rely on AMF mediated nutrient uptake strategy.

Acknowledgements

SC received funding from long term DAAD scholarship to carry out the research. ML is funded by the German Research Foundation (DFG; FOR 456, FOR 1451 – "The Jena Experiment") and by the "Zwillenberg-Tietz Stiftung". We acknowledge help from Agnes Fastnacht with greenhouse resources and monitoring of the experiment. Special thanks to Karl Kübler for construction and deployment of the pulse labelling setup in the greenhouse. We acknowledge Heike Geilmann and Steffen Ruehlow for help with stable isotope measurements, and Maria Foerster for helping with fatty acid analysis We also thank Prof. (Dr.) Erika Kothe, Ruchira Mukherji, Elisa Catao and Huei Ying Gan for helpful comments and discussions.

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

538 # References:

539 Anderson, I. C., and Parkin, P. I. (2007). Detection of active soil fungi by RT-PCR
540 amplification of precursor rRNA molecules. *J. Microbiol. Methods* 68, 248–253.
541 doi:10.1016/j.mimet.2006.08.005.

542 Averill, C., Bhatnagar, J. M., Dietze, M. C., Pearse, W. D., and Kivlin, S. N. (2019). Global
543 imprint of mycorrhizal fungi on whole-plant nutrient economics. *Proc. Natl. Acad. Sci.*
544 *U. S. A.* 116, 23163–23168. doi:10.1073/pnas.1906655116.

545 Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., et al.
546 (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal
547 ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing
548 data. *Methods Ecol. Evol.* 4, 914–919. doi:10.1111/2041-210X.12073.

549 Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification.
550 *Can. J. Biochem. Physiol.* 37, 911–917. doi:10.1139/y59-099.

551 Bonfante, P., and Genre, A. (2010). Mechanisms underlying beneficial plant-fungus
552 interactions in mycorrhizal symbiosis. *Nat. Commun.* 1, 48. doi:10.1038/ncomms1046.

553 Bowles, T. M., Jackson, L. E., and Cavagnaro, T. R. (2018). Mycorrhizal fungi enhance plant
554 nutrient acquisition and modulate nitrogen loss with variable water regimes. *Glob.*
555 *Chang. Biol.* 24, e171–e182. doi:10.1111/gcb.13884.

556 Brundrett, M. . (2015a). Arbuscular Mycorrhizas. In: Mycorrhizal Associations: The Web
557 Resource. Version 2.0. 24.04.2015.‹mycorrhizas.info›. Available at:
558 http://mycorrhizas.info/vam.html.

559 Brundrett, M. . (2015b). Mycorrhizal Associations: The Web Resource. Version 2.0.
560 24.04.2015. ‹mycorrhizas.info›. Available at: http://mycorrhizas.info/nmplants.html.

561 Bukovská, P., Bonkowski, M., Konvalinková, T., Beskid, O., Hujslová, M., Püschel, D., et al.
562 (2018). Utilization of organic nitrogen by arbuscular mycorrhizal fungi—is there a
563 specific role for protists and ammonia oxidizers? *Mycorrhiza* 28, 269–283.

564 doi:10.1007/s00572-018-0825-0.

565 Bunn, R. A., Simpson, D. T., Bullington, L. S., Lekberg, Y., and Janos, D. P. (2019).
566 Revisiting the 'direct mineral cycling' hypothesis: arbuscular mycorrhizal fungi colonize
567 leaf litter, but why? *ISME J.*, 1. doi:10.1038/s41396-019-0403-2.

568 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K.,
569 et al. (2010). QIIME allows analysis of high-throughput community sequencing data.
570 *Nat. Methods* 7, 335–336. doi:10.1038/nmeth.f.303.

571 Craine, J. M., Morrow, C., and Fierer, N. (2007). Microbial nitrogen limitation increases
572 decomposition. *Ecology* 88, 2105–2113. doi:10.1890/06-1847.1.

573 Davison, J., Moora, M., Opik, M., Adholeya, A., Ainsaar, L., Ba, A., et al. (2015). Global
574 assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism.
575 *Science* 349, 970–973. doi:10.1126/science.aab1161.

576 de Graaff, M.-A., Classen, A. T., Castro, H. F., and Schadt, C. W. (2010). Labile soil carbon
577 inputs mediate the soil microbial community composition and plant residue
578 decomposition rates. *New Phytol.* 188, 1055–1064. doi:10.1111/j.1469-
579 8137.2010.03427.x.

580 Dijkstra, F. A., Carrillo, Y., Pendall, E., and Morgan, J. A. (2013). Rhizosphere priming: a
581 nutrient perspective. *Front. Microbiol.* 4, 216. doi:10.3389/fmicb.2013.00216.

582 Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., et al.
583 (2010). Shifting carbon flow from roots into associated microbial communities in
584 response to elevated atmospheric CO_2. *Proc. Natl. Acad. Sci. U. S. A.* 107, 10938–42.
585 doi:10.1073/pnas.0912421107.

586 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
587 *Bioinformatics* 26, 2460–2461. doi:10.1093/bioinformatics/btq461.

588 Frostegård, A., and Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate
589 bacterial and fungal biomass in soil. *Biol. Fertil. Soils* 22, 59–65.

590 doi:10.1007/s003740050076.
591 Gleixner, G. (2013). Soil organic matter dynamics: a biological perspective derived from the
592 use of compound-specific isotopes studies. *Ecol. Res.* 28, 683–695. doi:10.1007/s11284-
593 012-1022-9.
594 Güsewell, S., and Gessner, M. O. (2009). N:P ratios influence litter decomposition and
595 colonization by fungi and bacteria in microcosms. *Funct. Ecol.* 23, 211–219.
596 doi:10.1111/j.1365-2435.2008.01478.x.
597 Gweon, H. S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D. S., et al. (2015). PIPITS:
598 An automated pipeline for analyses of fungal internal transcribed spacer sequences from
599 the Illumina sequencing platform. *Methods Ecol. Evol.* 6, 973–980. doi:10.1111/2041-
600 210X.12399.
601 Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V, Giannoukos, G., et al.
602 (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-
603 pyrosequenced PCR amplicons. *Genome Res.* 21, 494–504. doi:10.1101/gr.112730.110.
604 Harley, J. L., and Harley, E. L. (1987). A Checklist of Mycorrhiza in the British Flora. *New*
605 *Phytol.* 105, 1–102. doi:10.1111/j.1469-8137.1987.tb00674.x.
606 Herman, D. J., Firestone, M. K., Nuccio, E., and Hodge, A. (2012). Interactions between an
607 arbuscular mycorrhizal fungus and a soil microbial community mediating litter
608 decomposition. *FEMS Microbiol. Ecol.* 80, 236–247. doi:10.1111/j.1574-
609 6941.2011.01292.x.
610 Hestrin, R., Hammer, E. C., Mueller, C. W., and Lehmann, J. (2019). Synergies between
611 mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition.
612 *Commun. Biol.* 2, 233. doi:10.1038/s42003-019-0481-8.
613 Husson, O. (2013). Redox potential (Eh) and pH as drivers of soil/plant/microorganism
614 systems: A transdisciplinary overview pointing to integrative opportunities for
615 agronomy. *Plant Soil* 362, 389–417. doi:10.1007/s11104-012-1429-7.

Hütsch, B. W., Augustin, J., and Merbach, W. (2002). Plant rhizodeposition — an important source for carbon turnover in soils. *J. Plant Nutr. Soil Sci.* 165, 397. doi:10.1002/1522-2624(200208)165:4<397::AID-JPLN397>3.0.CO;2-C.

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. (2012). New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* 82, 666–677. doi:10.1111/j.1574-6941.2012.01437.x.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. *Front. Plant Sci.* 8, 1617. doi:10.3389/fpls.2017.01617.

Jilling, A., Keiluweit, M., Contosta, A. R., Frey, S., Schimel, J., Schnecker, J., et al. (2018). Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. *Biogeochemistry* 139, 103–122. doi:10.1007/s10533-018-0459-5.

Jones, D. L., Hodge, A., and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163, 459–480. doi:10.1111/j.1469-8137.2004.01130.x.

Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., et al. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. *New Phytol.* 205. doi:10.1111/nph.13138.

Kallenbach, C. M., Frey, S. D., and Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nat. Commun.* 7, 13630. doi:10.1038/ncomms13630.

Karlowsky, S., Augusti, A., Ingrisch, J., Hasibeder, R., Lange, M., Lavorel, S., et al. (2018). Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. *J. Ecol.* 106, 1230–1243. doi:10.1111/1365-2745.12910.

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., and Kleber, M.

642 (2015). Mineral protection of soil carbon counteracted by root exudates. *Nat. Clim.*
643 *Chang.* 5, 588–595. doi:10.1038/nclimate2580.
644 Klamer, M. (2004). Estimation of conversion factors for fungal biomass determination in
645 compost using ergosterol and PLFA 18:2ω6,9. *Soil Biol. Biochem.* 36, 57–65.
646 doi:10.1016/j.soilbio.2003.08.019.
647 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013).
648 Development of a dual-index sequencing strategy and curation pipeline for analyzing
649 amplicon sequence data on the miseq illumina sequencing platform. *Appl. Environ.*
650 *Microbiol.* 79, 5112–5120. doi:10.1128/AEM.01043-13.
651 Kuzyakov, Y., and Xu, X. (2013). Competition between roots and microorganisms for
652 nitrogen: mechanisms and ecological relevance. *New Phytol.* 198, 656–69.
653 doi:10.1111/nph.12235.
654 Levin, P. A., and Angert, E. R. (2015). Small but mighty: Cell size and bacteria. *Cold Spring*
655 *Harb. Perspect. Biol.* 7, 1–11. doi:10.1101/cshperspect.a019216.
656 Liang, C., Schimel, J. P., and Jastrow, J. D. (2017). The importance of anabolism in microbial
657 control over soil carbon storage. *Nat. Microbiol.* 2, 17105.
658 doi:10.1038/nmicrobiol.2017.105.
659 Malik, A. A., Dannert, H., Griffiths, R. I., Thomson, B. C., and Gleixner, G. (2015).
660 Rhizosphere bacterial carbon turnover is higher in nucleic acids than membrane lipids:
661 Implications for understanding soil carbon cycling. *Front. Microbiol.* 6, 268.
662 doi:10.3389/fmicb.2015.00268.
663 Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., et al.
664 (2018). Land use driven change in soil pH affects microbial carbon cycling processes.
665 *Nat. Commun.* 9, 1–10. doi:10.1038/s41467-018-05980-1.
666 McLaren, J. R., and Turkington, R. (2011). Plant identity influences decomposition through
667 more than one mechanism. *PLoS One* 6, e23702. doi:10.1371/journal.pone.0023702.

668 Meier, I. C., Pritchard, S. G., Brzostek, E. R., McCormack, M. L., and Phillips, R. P. (2015).
669 The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling
670 in forests exposed to elevated CO_2. *New Phytol.* 205, 1164–74. doi:10.1111/nph.13122.
671 Mueller, R. C., Balasch, M. M., and Kuske, C. R. (2014). Contrasting soil fungal community
672 responses to experimental nitrogen addition using the large subunit rRNA taxonomic
673 marker and cellobiohydrolase I functional marker. *Mol. Ecol.* 23, 4406–17.
674 doi:10.1111/mec.12858.
675 Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016).
676 FUNGuild: An open annotation tool for parsing fungal community datasets by ecological
677 guild. *Fungal Ecol.* 20, 241–248. doi:10.1016/J.FUNECO.2015.06.006.
678 Nuccio, E. E., Hodge, A., Pett-Ridge, J., Herman, D. J., Weber, P. K., and Firestone, M. K.
679 (2013). An arbuscular mycorrhizal fungus significantly modifies the soil bacterial
680 community and nitrogen cycling during litter decomposition. *Environ. Microbiol.* 15,
681 1870–1881. doi:10.1111/1462-2920.12081.
682 Nuccio, E. E., Starr, E., Karaoz, U., Brodie, E. L., Zhou, J., Tringe, S. G., et al. (2020). Niche
683 differentiation is spatially and temporally regulated in the rhizosphere. *ISME J.* 14, 999–
684 1014. doi:10.1038/s41396-019-0582-x.
685 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2018).
686 vegan: Community Ecology Package. Available at: https://cran.r-
687 project.org/package=vegan.
688 Rodriguez, R. J., White Jr, J. F., Arnold, A. E., and Redman, R. S. (2009). Fungal
689 endophytes: diversity and functional roles. *New Phytol.* 182, 314–330.
690 doi:10.1111/j.1469-8137.2009.02773.x.
691 Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile
692 open source tool for metagenomics. *PeerJ* 4, e2584. doi:10.7717/peerj.2584.
693 Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., et al.

(2004). The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic Appl. Ecol.* 5, 107–121. doi:10.1078/1439-1791-00216.

Sanders, F. E., and Tinker, P. B. (1973). Phosphate flow into mycorrhizal roots. *Pestic. Sci.* 4, 385–395. doi:10.1002/ps.2780040316.

Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* 85, 591–602. doi:10.1890/03-8002.

Shi, S., Nuccio, E. E., Shi, Z. J., He, Z., Zhou, J., and Firestone, M. K. (2016). The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecol. Lett.* 19, 926–936. doi:10.1111/ele.12630.

Stempfhuber, B., Richter-Heitmann, T., Regan, K. M., Kölbl, A., Wüst, P. K., Marhan, S., et al. (2016). Spatial interaction of archaeal ammonia-oxidizers and nitrite-oxidizing bacteria in an unfertilized grassland soil. *Front. Microbiol.* 6, 1567. doi:10.3389/fmicb.2015.01567.

Strickland, M. S., Osburn, E., Lauber, C., Fierer, N., and Bradford, M. A. (2009). Litter quality is in the eye of the beholder: Initial decomposition rates as a function of inoculum characteristics. *Funct. Ecol.* 23, 627–636. doi:10.1111/j.1365-2435.2008.01515.x.

Thomson, B. C., Ostle, N. J., McNamara, N. P., Oakley, S., Whiteley, A. S., Bailey, M. J., et al. (2013). Plant soil interactions alter carbon cycling in an upland grassland soil. *Front. Microbiol.* 4, 253. doi:10.3389/fmicb.2013.00253.

Thuille, A., Laufer, J., Höhl, C., and Gleixner, G. (2015). Carbon quality affects the nitrogen partitioning between plants and soil microorganisms. *Soil Biol. Biochem.* 81, 266–274. doi:10.1016/j.soilbio.2014.11.024.

Tisserant, E., Malbreil, M., Kuo, A., Kohler, A., Symeonidi, A., Balestrini, R., et al. (2013). Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant

symbiosis. *Proc. Natl. Acad. Sci.* 110, 20117–20122. doi:10.1073/pnas.1313452110.

Veresoglou, S. D., Chen, B., and Rillig, M. C. (2012). Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol. Biochem.* 46, 53–62. doi:10.1016/J.SOILBIO.2011.11.018.

Vestergård, M., Henry, F., Rangel-Castro, J. I., Michelsen, A., Prosser, J. I., and Christensen, S. (2008). Rhizosphere bacterial community composition responds to arbuscular mycorrhiza, but not to reductions in microbial activity induced by foliar cutting. *FEMS Microbiol. Ecol.* 64, 78–89. doi:10.1111/j.1574-6941.2008.00447.x.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–7. doi:10.1128/AEM.00062-07.

Weisser, W. W., Roscher, C., Meyer, S. T., Ebeling, A., Luo, G., Allan, E., et al. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic Appl. Ecol.* 23, 1–73. doi:10.1016/j.baae.2017.06.002.

Whiteside, M. D., Werner, G. D. A., Caldas, V. E. A., van't Padje, A., Dupin, S. E., Elbers, B., et al. (2019). Mycorrhizal Fungi Respond to Resource Inequality by Moving Phosphorus from Rich to Poor Patches across Networks. *Curr. Biol.* 29, 2043-2050.e8. doi:10.1016/J.CUB.2019.04.061.

Wickham, H. (2011). ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* 3, 180–185. doi:10.1002/wics.147.

Williams, A., Manoharan, L., Rosenstock, N. P., Olsson, P. A., and Hedlund, K. (2017). Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (*Hordeum vulgare*) mycorrhizal carbon and phosphorus exchange. *New Phytol.* 213, 874–885. doi:10.1111/nph.14196.

Xu, X., Thornton, P. E., and Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* 22,

746 737–749. doi:10.1111/geb.12029.

747 Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., et al. (2018).
748 Dynamic root exudate chemistry and microbial substrate preferences drive patterns in
749 rhizosphere microbial community assembly. *Nat. Microbiol.* 3, 470–480.
750 doi:10.1038/s41564-018-0129-3.

751 Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). PEAR: a fast and accurate
752 Illumina Paired-End reAd mergeR. *Bioinformatics* 30, 614–20.
753 doi:10.1093/bioinformatics/btt593.

754 Zhu, Q., Riley, W. J., and Tang, J. (2017). A new theory of plant-microbe nutrient
755 competition resolves inconsistencies between observations and model predictions. *Ecol.*
756 *Appl.* 27, 875–886. doi:10.1002/eap.1490.

757

Figures and Tables Legends

Figure 1: Experimental setup of the study.

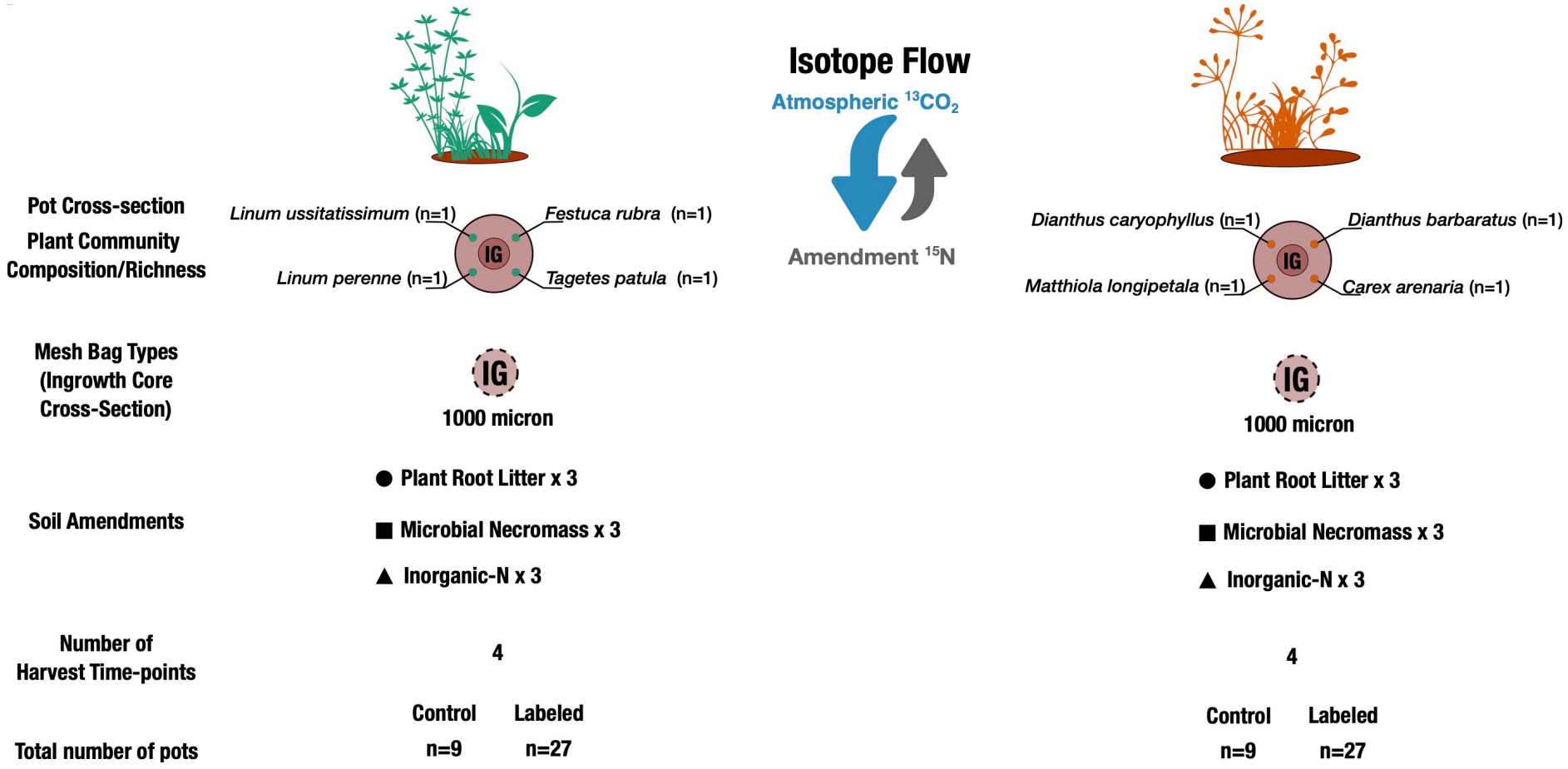


Figure 2: Photosynthetically fixed carbon assimilated by plants and associated microbial community. A: Comparison of root to shoot ^{13}C content between AM (n = 9) and NM (n = 9) the plant community types across all soil amendments. **B:** Mean ^{13}C (ng) excess in soil microbial biomass of each plant community types. Height of the bars represents mean and error bars represent standard error of mean. Different letters on bars designate significant differences based on one way analysis of variance (ANOVA) comparing plant community types. Colors represent plant community types where Dark Green=AM plant community and Orange= NM plant community.

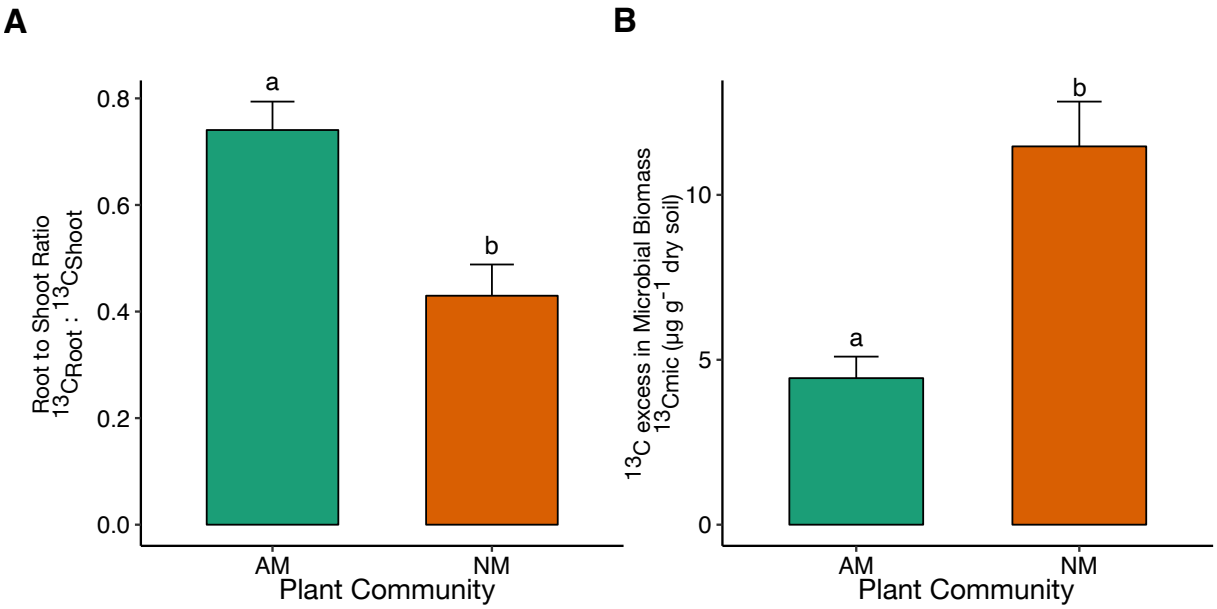


Figure 3: Carbon investment into microbial community and return of decomposition released nitrogen. A: ^{15}N content (µg) of shoot biomass compared between AM (n = 9) and NM (n = 9) for plant communities. **B:** Comparison of carbon investment by plant communities into total microbial biomass as determined from ^{13}C excess in total microbial biomass (PLFA) scaled up to ingrowth core soil weight (200 g) per µg for respective plant communities. Colors represent plant community types (Dark Green = AM and Orange = NM plant community). In both cases, soil amendments were aggregated for each plant community. Horizontal bars represent means for each group and error bars represent standard error of mean across all soil amendment for the specific plant community. Groups sharing the same letters are not significantly different based on one-way analysis of variance (ANOVA) comparing only plant communities.

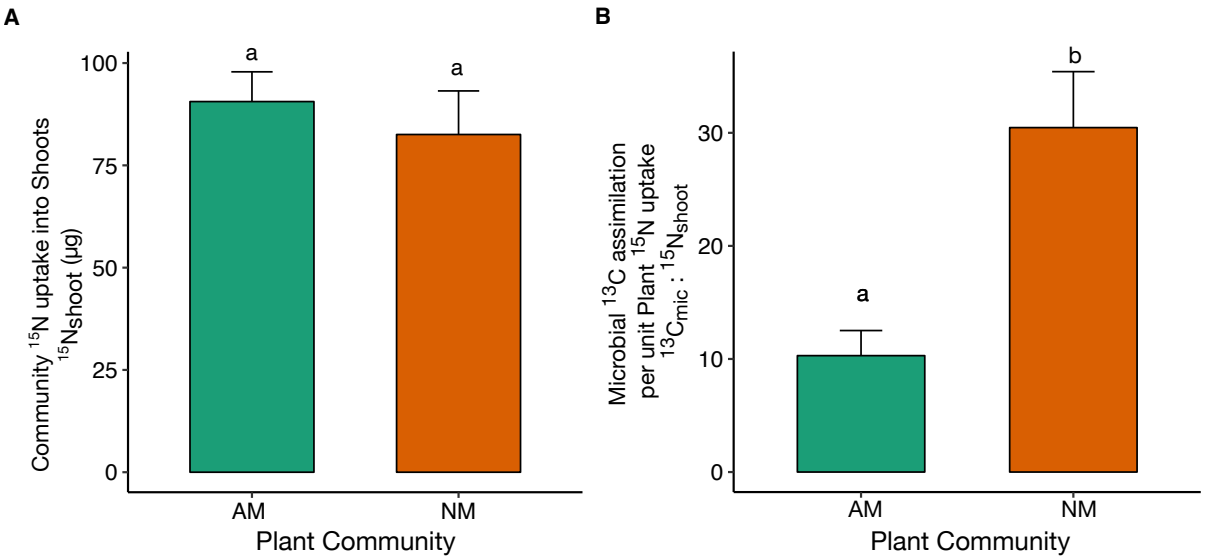


Figure 4: ^{13}C investment for ^{15}N gain by plants partitioned based on microbial groups

Mean ^{13}C incorporated into total microbial group specific lipids (A – AMF (NLFA) ; B – Free-living fungi (PLFA) and C – Bacteria (PLFA)) per unit ^{15}N uptake by the plant community. Standard error calculated based on replicated plant community ^{15}N uptake measurements (n = 3 per soil amendment nested under plant community type). Means, standard error and significant differences between plant communities (asterisk) and soil amendments (LSD letter-group based on Tukey's post-hoc tests) are shown. Significance is indicated at $p < 0.05$ (*) , $p < 0.01$ (**) and $p < 0.001$ (***) .

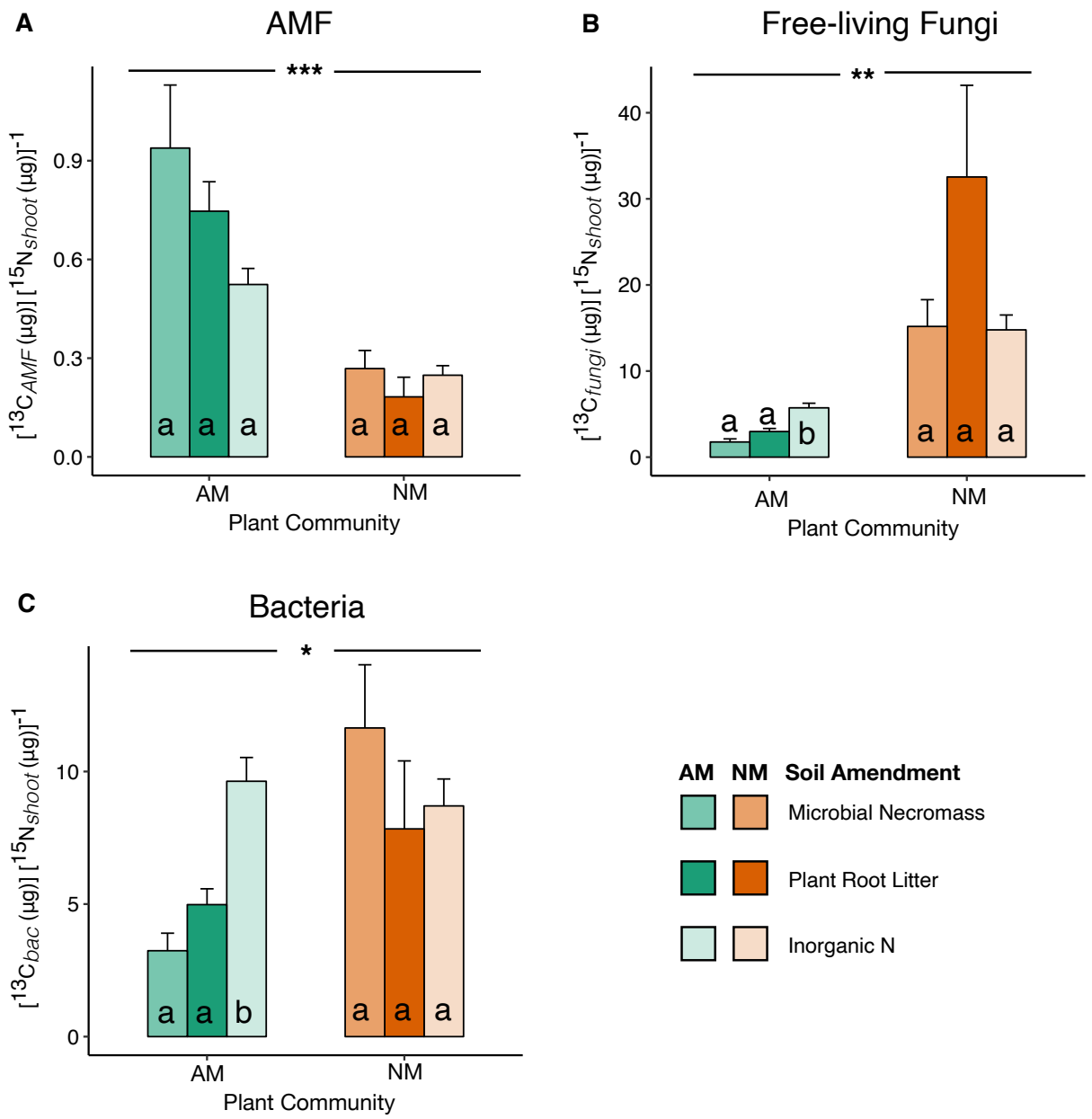
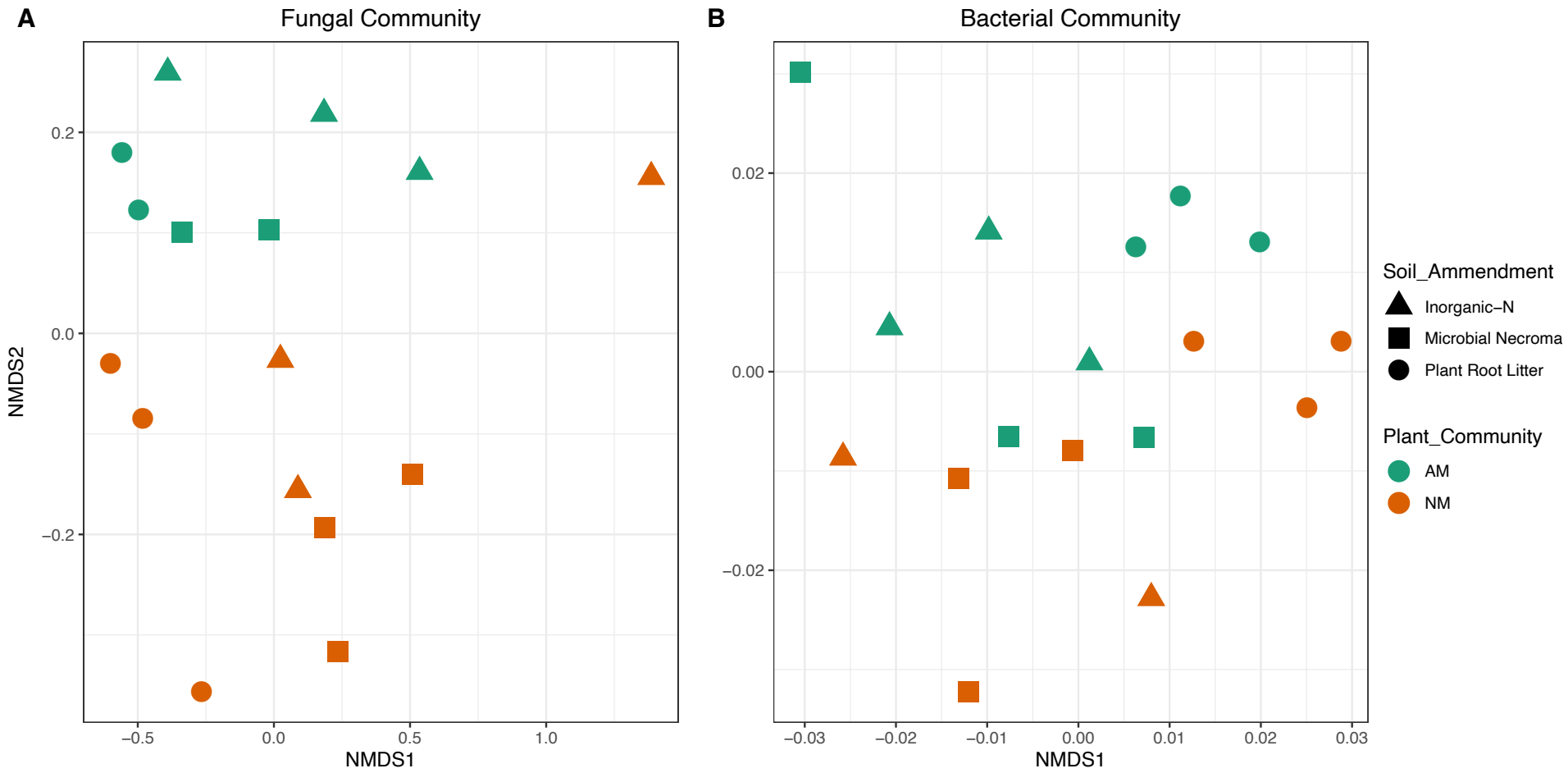


Figure 5: Variation in microbial bacterial and fungal community composition

Non-metric multidimensional scaling (NMDS) plots illustrating Bray-Curtis distances between microbial communities (A: Fungal Community and B: Bacterial Community) in individual samples. Colors represent plant community type (Dark Green = AM and Orange = NM) whereas symbols represents soil amendments. Stress values for fungal (B) and bacterial (A) plots are 0.05 and 0.18 respectively.


A
Fungal Community
NMDS2
NMDS1
0.2
0.0
−0.2
−0.5
0.0
0.5
1.0
B
Bacterial Community
NMDS1
0.02
0.00
−0.02
−0.03
−0.02
−0.01
0.00
0.01
0.02
0.03
Soil_Ammendment
Inorganic−N
Microbial Necroma
Plant Root Litter
Plant_Community
AM
NM

806

807

808

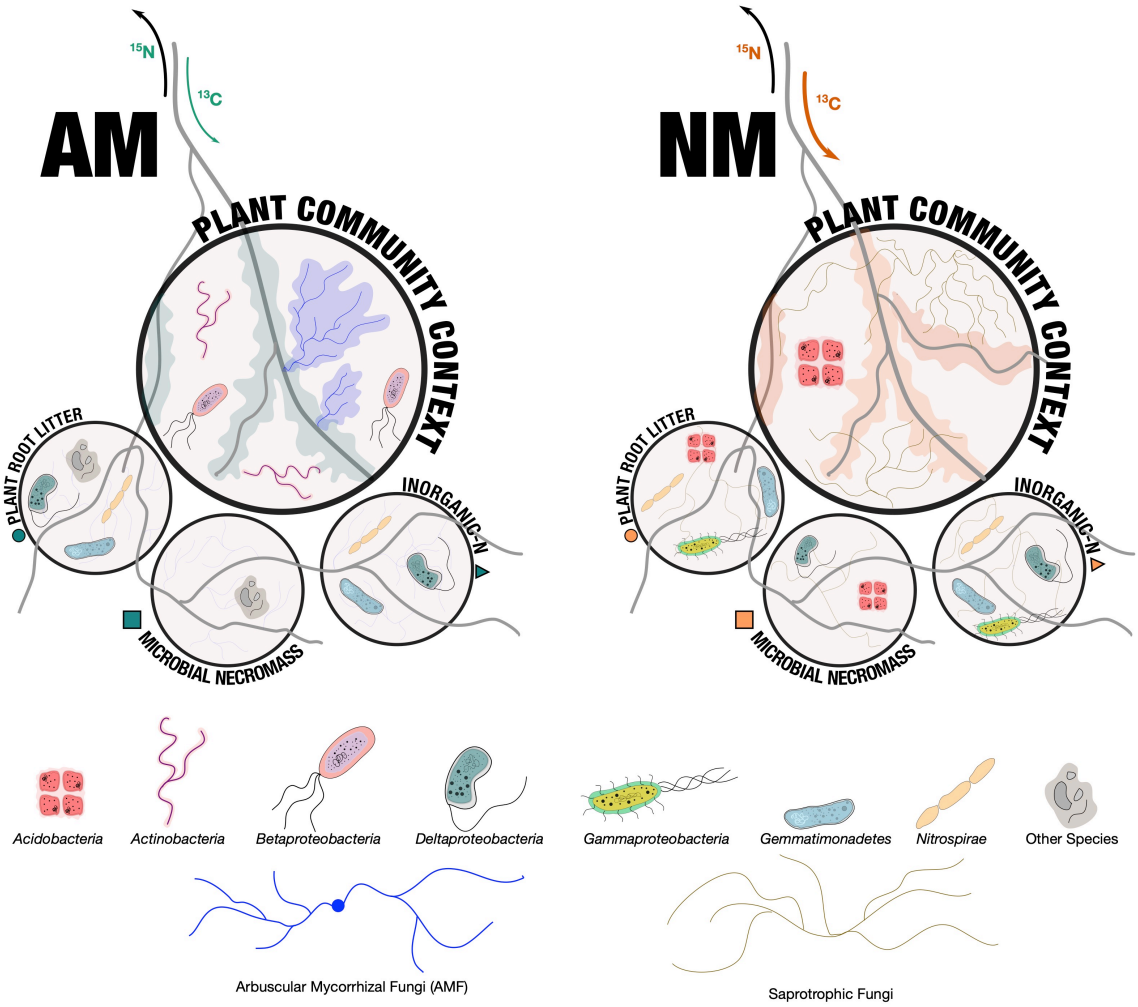



Figure 6 – Conceptual model showing the effect of AMF association on decomposer and plant responsive microbial groups that shape carbon and nutrient flux when decomposing stoichiometrically identical substrates. In non-mycorrhizal plants (NM) the role of AMF is taken over by fungal saprotrophs. Distinct bacterial communities are assembled for each substrate depending on substrate origin and plant-AMF association context. Arrows represent the C and N flow differences arising from presence or absence of AMF, explaining that for an equivalent N uptake AMF associated plants invested lower amounts of photoassimilated carbon into decomposer community.