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Abstract: Plant resource partitioning is a mechanism promoting species coexistence and ecosystem functioning. Yet, we still have limited understanding of how soil microbes, especially plant symbiotic microbes, influence resource partitioning. We hypothesized that soil-borne microbes, in particular mycorrhizal fungi, facilitate differential performance of tree species depending on different nitrogen sources and that this leads to a positive plant diversity–community productivity relationship. We conducted two complementing glasshouse experiments. In a "monoculture experiment," we supplied nitrogen as ammonium, nitrate or glycine and tested the growth response of three tree species associated with different root symbionts: one associated with ectomycorrhizal fungi, one associated with arbuscular mycorrhizal fungi, and the third associated with both arbuscular mycorrhizal fungi and N-fixing bacteria. In an "intermixed experiment," we grew the tree species at three richness levels (one, two or three species) in soil supplied with a mix of the three nitrogen forms or no added nitrogen, and with or without soil microbes. The monoculture experiment showed that in the presence of soil microbes, the ectomycorrhizal plant species grew best when supplied with glycine and the two arbuscular mycorrhizal plant species grew best with either nitrate or ammonium addition. When the different forms of nitrogen were mixed in the intermixed experiment, plant mixtures produced more biomass than plant monocultures in the presence of soil microbes, with positive complementarity effects indicating microbe-mediated plant resource partitioning. Our results suggest that co-existing tree species can partition soil nitrogen when grown with their particular mycorrhizal symbionts or other soil microbes, resulting in positive biodiversity effects in complex resource environments.

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Soil microbes promote complementarity effects among co-existing trees through soil nitrogen partitioning

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Running title: Microbe-mediated plant N partitioning

Abstract

1. Plant resource partitioning is a mechanism promoting species coexistence and ecosystem functioning. Yet, we still have limited understanding of how soil microbes, especially plant symbiotic microbes, influence resource partitioning. We hypothesized that soil borne microbes, in particular mycorrhizal fungi, facilitate differential performance of tree species depending on different nitrogen sources and that this leads to a positive plant diversity–community productivity relationship.

2. We conducted two complementing glasshouse experiments. In a "monoculture experiment", we supplied nitrogen as ammonium, nitrate or glycine and tested the growth response of three tree species associated with different root symbionts: one associated with ectomycorrhizal fungi, one associated with arbuscular mycorrhizal fungi and the third associated with both arbuscular mycorrhizal fungi and N-fixing bacteria. In an "intermixed experiment", we grew the tree species at three richness levels (one, two or three species) in soil supplied with a mix of the three nitrogen forms or no added nitrogen, and with or without soil microbes.

3. The monoculture experiment showed that in the presence of soil microbes, the ectomycorrhizal plant species grew best when supplied with glycine and the two arbuscular mycorrhizal plant species grew best with either nitrate or ammonium addition. When the different forms of nitrogen were mixed in the intermixed experiment, plant mixtures produced more biomass than plant monocultures in the presence of soil microbes, with positive complementarity effects indicating microbe-mediated plant resource partitioning.

4. Our results suggest that co-existing tree species can partition soil nitrogen when grown with their particular mycorrhizal symbionts or other soil microbes, resulting in positive biodiversity effects in complex resource environments.

Key-words: Biodiversity, complementarity, ecosystem functioning, mycorrhizal fungi, nitrogen partitioning, plant–soil interactions, soil microbes

Introduction

Plant niche partitioning is considered to promote species coexistence and complementary resource use, providing a mechanistic explanation for positive biodiversity effects on ecosystem primary productivity in biodiversity–ecosystem functioning experiments (Tilman 1982; Tilman, Lehman, & Thomson 1997; Loreau & Hector, 2001; Levine & HilleRisLambers, 2009). Functionally dissimilar species may occupy complementary ecological niches, for example by specializing on different chemical forms of the same elemental nutrient (McKane et al., 2002; Ceulemans et al., 2017). Such specialized plant nutrient uptake may be enabled by different associations with symbiotic microbes, in particular mycorrhizal fungi (Phillips, Brzostek, & Midgley 2013). Symbiotic microbes can expand the niche of plant species through increasing plant nutrient uptake rates or altering the plant preference for specific nutrient forms (van der Heijden et al., 1998; Márquez, Redman, Rodriguez, & Roossinck 2007; Wu et al., 2013; Afkhami, McIntyre, & Strauss 2014). For instance, mycorrhiza can promote the growth of host plants in soils with low levels of plant-available phosphorus (Schweiger & Jakobsen 1999). In addition, experimental grassland studies found that diverse arbuscular mycorrhizal fungal communities increased plant community productivity (van der Heijden et al., 1998; Maherali & Klironomos, 2007; Wagg, Barendregt, Jansa, & van der Heijden 2015). These results further suggest that mycorrhiza can have positive effects on plant community as a whole by expanding community niche, as predicted by a theoretical study of Poisot, Mouquet and Gravel (2013). However, we still have a limited understanding as to whether the

combination of different types of mycorrhizal fungi and other soil microbes mediates resource partitioning among plant species and whether this can lead to greater plant complementarity in diverse communities.

Arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi are the two most common types of mycorrhiza; they form symbioses with about 80% of all terrestrial plant species and can help plant nutrient acquisition, especially nitrogen (N) and phosphorus (P) (Lambers, Raven, Shaver, & Smith 2008; Smith & Read, 2008; Brundrett, 2009; van der Heijden, Martin, Selosse, & Sanders 2015). However, these two types of mycorrhiza are functionally distinct with respect to N-acquisition (Taylor & Alexander, 2005; Smith & Read, 2008). EM fungi are capable of producing extracellular enzymes that degrade complex organic N and take up dissolved organic N such as amino acids (Chalot & Brun, 1998; Endo, Norisada, Kogawara, Hogetsu, & Kojima 2009; Courty et al., 2010; Li et al., 2016). Unlike EM fungi that have access to organic N, AM fungi have no known saprotrophic capacity and function as an extension of roots to acquire inorganic N (Smith & Read, 2008; Smith & Smith, 2011; but see Hodge, Campbell, & Fitter 2001; Leigh, Hodge, & Fitter 2009). It has been shown that AM fungi can mobilize soil nitrate and transfer it to plant root cells (Azćon, Gomez, & Tobar 1996; Azćon & Tobar, 1998; Liu et al., 2017). Thus, it is possible that tree species associated with either AM and EM fungi form complementary N-uptake niches that may promote their combined resource uptake, growth and productivity in forests.

Here we test the hypothesis that plant nutrient partitioning arises from differential associations of plant species with microbial symbionts that are able to access different

forms of soil nutrients (Bever et al., 2010; Reynolds, Packer, Bever, & Clay 2003). The few attempts to test this hypothesis have focused on grassland ecosystems dominated by a single mycorrhizal type (i.e. AM fungi) and have failed to support it (Reynolds, Hartley, Vogelsang, Bever, & Schultz 2005; Vogelsang, Reynolds, & Bever 2006). Additional tests with plants in other systems such as forests, where some trees are associated with EM fungi and others with AM fungi, and provided with mixed forms of soil nutrients, are needed to evaluate whether and how mycorrhiza and other root-associated soil microbes contribute to plant resource partitioning.

To test our hypothesis, we set up two complementing glasshouse experiments. In a "monoculture experiment", we supplied N as ammonium, nitrate or glycine and measured the corresponding growth response of three tree species associated with different symbiotic partners (AM fungi, EM fungi or dual AM fungi and N-fixing bacteria). We predicted that species associated with EM fungi grow best on glycine, species with AM fungi grow best on nitrate, and species associated with both AM fungi and N-fixing bacteria grow best on ammonium, as nitrate can inhibit symbiotic nitrogen fixation (Voisin, Salon, Munier-Jolain, & Ney 2002). In an "intermixed experiment", we grew the tree species at three richness levels (one, two or three species) in soil supplied with a mixture of the three N forms or no added N, and with or without soil microbes. Our goal was to assess whether a positive plant species richness–community productivity relationship can be promoted by microbial inoculation of an environment with multiple forms of soil N. Finally, we tested whether plant community productivity can be predicted by their "community niche", which represents the potential for

112 complementary resource use in a community (Salles, Poly, Schmid, & Roux 2009).

113 Our experiments address two topics. First, our test of microbe-mediated plant N
114 partitioning contributes to our understanding of the mechanisms underlying plant
115 resource partitioning. Although evidence for plant partitioning of chemical N forms is
116 accumulating in arctic tundra, alpine meadows and temperate grassland, these studies
117 did not test whether N was directly taken up by plant roots or via symbiotic microbes
118 (McKane et al., 2002; Miller & Bowman, 2002; Weigelt, Bol, & Bardgett 2005; Miller,
119 Bowman, & Suding 2007). Second, our experiments step further to look at the
120 ecosystem consequence of microbe-mediated plant N partitioning. Previous studies of
121 plant N partitioning used short-term stable isotope methods, which raises the question
122 whether the observed differences in N uptake are representative of long-term use of N
123 that facilitates plant growth and community productivity.

124

125 **Materials and Methods**

126 ***Study site and species***

127 We conducted the glasshouse experiments at Heishiding Nature Reserve
128 (111°53'E, 23°27'N; 150–927 m altitude) in Guangdong Province, China. The reserve
129 has a subtropical moist monsoon climate. Mean annual temperature is 19.6℃, with a
130 high mean temperature of 28.4℃ in July and a mean low temperature of 10.6℃ in
131 January.

132 We chose three commonly co-occurring tree species with different mycorrhizal
133 associations: one EM species, *Lithocarpus litseifolius* (Hance) Chun (Fagaceae), and

two AM species, *Cryptocarya concinna* Hance (Lauraceae) and *Ormosia glaberrima* Y. C. Wu (Fabaceae). *Ormosia glaberrima* is also a N-fixing species. We collected seeds of these focal species during the autumn and winter of 2013. We surface-sterilized the seeds (1 min 70% ethanol, 3 min 2.625% NaOCl, 1 min 70% ethanol, and 1 min distilled water), and stored them at 4℃ until March 2014. From March to April, all seeds were geminated in plastic boxes filled with sterilized sand.

Background soil characteristics

In June 2014, we collected loamy clay soil from the forest field. The soil had high capacities for holding water and nutrients. The soil was mixed with sand (1:2 v:v), sterilized by gamma radiation (25 KGy) and used as background soil. We used a high proportion of sand to reduce the nutrient availability of the background soil, which promoted plants to use the supplied N sources. In the background soil (pH = 4.47), the ammonium concentration was 4.99 mg kg^{-1}, the nitrate concentration was 29.7 mg kg^{-1}, the concentration of available P was 2.17 mg kg^{-1}, and the percentage organic matter was 0.55%.

Monoculture experiment testing for the growth response of tree species to different forms of N

The monoculture experiment had a factorial design that each of the three species grew with unsterilized or sterilized soil inoculum and was supplied with each one of the different forms of N (ammonium, nitrate or glycine addition). Each treatment was

156 replicated 15 times yielding a total of 270 pots (3 species × 2 soil-inoculum treatments
157 × 3 N treatments × 15 replicates). We filled pots of 6 cm diameter and 11 cm height
158 with 200 ml of the background soil, with each pot receiving either unsterilized or
159 sterilized soil inoculum. One newly germinated seedling was transplanted into each pot.
160 The transplanted seedlings were similar in size to help reduce any seedling size-
161 dependent effects that could be introduced prior to the beginning of the experiment.

162 To prepare unsterilized or sterilized soil inoculum, we collected living soil
163 beneath adults of all focal species at the same forest field site, with three replicated
164 adults for each species. Living soil samples of the same species were then mixed
165 thoroughly and divided into two halves. The first half was sterilized by gamma
166 radiation and the second half was used as unsterilized soil inoculum. For the
167 unsterilized soil-inoculum treatment, we added 10 ml of living soil onto the
168 background soil in each pot, covered by another 10 ml of sterilized soil to prevent
169 cross-infection among pots. For the sterilized soil-inoculum treatment, we added 20 ml
170 of sterilized soil into each pot as a layer on top of the background soil. Together with
171 the original 200 ml of background soil, each pot contained 220 ml (277.4 ± 2.7 g dry
172 weight) of soil.

173 One month after the planting of seedlings, each species was treated with
174 ammonium (NH_4^+, as $(NH_4)_2SO_4$), nitrate (NO_3^-, as KNO_3), or amino acid glycine
175 (NH_2CH_2COOH). Nitrogen treatments were applied in solution forms (20 ml per pot)
176 every month during the experiment at 100 mg N l^{-1}, a relatively low concentration (7.21
177 mg kg^{-1}) designed to ensure full uptake of the supplied N by plants, which according to

our observation was not stressful for plant growth. We intermittently stopped watering the plants four days before each N treatment without causing any drought symptoms to reduce soil moisture and prevent the outflow of added N. In total, 28 mg N was added per pot during the experimental period. We placed all pots in 15 blocks, within which pots were arranged in random positions.

Intermixed experiment testing for the response of communities to mixed N forms

Using the same set of species as in the monoculture experiment, we assembled plant communities into pots at three species richness levels (one, two or three species) in the intermixed experiment. Specifically, each of the three species was planted as a monoculture (richness level of one). All three possible two-species mixtures and the three-species mixture were planted with the species in equal portions and the same total density as the monocultures, namely six individuals per pot (substitutive design). Each community composition grew in three different soil conditions: 1) unsterilized or 2) sterilized soil supplied with a mix of the three N forms used separately in the monoculture experiment or 3) unsterilized soil without N addition. Each experimental unit had 10 replicates, in total there were 210 pots (7 assemblages × 3 soil conditions × 10 replicates). We filled pots of 12 cm diameter and 13 cm height with 1000 ml of the background soil as used in the monoculture experiment, with each pot receiving either unsterilized or sterilized soil inoculum. Six newly germinated seedlings were transplanted into each pot. In total, there were 1260 tree seedlings (210 pots × 6 seedlings).

To prepare the unsterilized and sterilized soil inoculum, we followed the same procedure as in the monoculture experiment, with the exception that we mixed all living soil samples. For the unsterilized soil-inoculum treatment, we added 50 ml of living soil onto the background soil in each pot, covered by another 50 ml of sterilized soil. For the sterilized soil-inoculum treatment, we added 100 ml of sterilized soil into each pot as a layer on top of the background soil. Together with the original 1000 ml of background soil, each pot contained 1100 ml (1363.2 ± 10.8 g dry weight) of soil. For the N-addition treatment, a mixture of all three N forms was applied monthly as a solution (50 ml per pot) at 200 mg N l^{-1}. The concentration of added N (7.34 mg kg^{-1}) in the intermixed experiment was similar to that of the monoculture experiment (7.21 mg kg^{-1}). In total, 140 mg N was added per pot for the N-addition treatment during the experiment. The control pots received the same amount of water as was supplied to the pots with N addition. There were 10 blocks, within which the pots with the different treatment combinations were arranged in random positions.

For both experiments, plants were watered twice a week between nutrient applications and allowed to grow for 14 months. Then we harvested the plants and determined the dry weight of shoots and roots for every individual.

Community niche

The niche of a given community (community niche, CN) was calculated based on the performance of each species on each N form in the presence of soil microbes, according to the formula:

$$CN = \sum_{i=1}^{3} \max_{j=1}^{n}(P_{ij}) \quad \text{(eqn 1)}$$

where P_{ij} is the best performance (here we used biomass) of species j on N form i, and n is the number of species in the considered community (Salles et al., 2009). The calculated community niche corresponds to the sum of the best performances of each species across the different N forms.

Mycorrhizal root colonization

To determine the effectiveness of mycorrhizal fungi inoculation using live soil inoculum and soil sterilization, we measured mycorrhizal colonization of roots sampled from both unsterilized and sterilized soil-inoculum treatments. As the percentage of soil inoculum that distinguished the unsterilized and sterilized soil treatments in each pot remained the same for the monoculture and intermixed experiments, we only sampled roots in the monoculture experiment. We acknowledge that this sampling method may neglect the potential effects of plant competition on mycorrhizal root colonization in the intermixed experiment, but at least it reflects the direct effect of soil-inoculum treatments on mycorrhizal root colonization in both experiments.

For each species, we collected five root samples for each soil-inoculum treatment. To evaluate the level of AM colonization, representative root samples were stained using the ink and vinegar staining technique (Vierheilig, Coughlan, Wyss, & Piché 1998). We assessed colonization of 10 randomly chosen 2-cm root sections per sample using the gridline intersection method (Giovannetti & Mosse, 1980). For each of the 2-

243 cm root sections, we checked 20 intersections. In total, we checked 200 intersections
244 for each sample (20 × 10). We scored vesicles, arbuscules and AM hyphae as positive
245 AM encounters. For each sample, the percentage of infection was calculated as the ratio
246 of intersections that contained AM fungal structures to the total number of intersections
247 observed.

248 The level of EM colonization was determined on representative root samples and
249 based on counts of all root tips present in the counting dish. We also had 10 randomly
250 chosen 2-cm root sections per sample. The presence of Hartig net and fungal mantle
251 distinguished EM from non-EM root tips (Gehring & Whitham, 1991). Therefore, all
252 root tips (non-EM and EM tips) were counted until 100 EM or 100 non-EM was reached
253 (Teste, Karst, Jones, Simard, & Durall 2006). Percentage of EM colonization was
254 calculated as the number of active EM root tips divided by the total number of root tips.

255

256 ***Statistical analyses***

257 *Plant species-specific growth response to different N forms in the monoculture*
258 *experiment*

259 We tested whether different tree species grew differently on the three N forms and
260 whether this depended on soil inoculum. We used a general linear model with the total
261 of above- and belowground biomass (log-transformed) of plant individuals as
262 dependent variable, and plant species identity, N treatments (ammonium, nitrate or
263 glycine addition), soil-inoculum treatments (unsterilized or sterilized inoculum) and
264 their interactions as explanatory variables.

Further, we tested whether each tree species grew best on a different form of N, as predicted in our hypothesis: *L. litseifolius* with EM fungi was expected to grow best on organic N, *C. concinna* with AM fungi on nitrate, and *O. glaberrima* with AM fungi and N-fixing bacteria on ammonium. We modified the above model by using the contrast "home" within the species identity × N treatments interaction, calling those species-by-N treatment combinations "home" that corresponded to the predicted N forms with which each tree species was expected to grow best (see Table 1 and Joshi et al., 2001 for how to apply "home" contrasts to interactions). The corresponding three-way interaction with soil-inoculum treatments was then used to test if the "home" effect was stronger in unsterilized soil, which would support the hypothesis that soil microbes are responsible for the different responses of tree species to different N forms.

Effects of soil inoculum on plant species richness–community productivity relationships and plant biodiversity effects

We split the data of the intermixed experiment into two parts, namely with or without N addition, and analyzed them separately. For the first part with N addition, we tested how community composition (the three monocultures, three 2-species mixtures and one 3-species mixture) — partitioned into a linear contrast of plant species richness and remaining community composition (for detailed explanation of this approach see Schmid, Baruffol, Wang, & Niklaus 2017) — and soil-inoculum treatments (unsterilized or sterilized inoculum) affected community productivity (the total of plant above- and belowground biomass) with an overall general linear model. Furthermore,

287 we analyzed the community productivity with N addition but with unsterilized or
288 sterilized soil inoculum separately using general linear models. For the second part
289 without N addition, similarly, we analyzed the responses of community productivity to
290 community composition using a general linear model. In all analyses we included block
291 as a fixed-effects term at the beginning of the model (Schmid et al., 2017). Because we
292 treated community composition as a fixed-effects term our inferences about
293 biodiversity effects are restricted to the particular species composition investigated.
294 Moreover, we tested whether soil-inoculum treatments (unsterilized or sterilized
295 inoculum) affected plant biodiversity effects when supplied with N. We used the
296 additive partitioning method of Loreau and Hector (2001) to partition net biodiversity
297 effects (NEs) into complementarity (CEs) and selection effects (SEs). We used similar
298 general linear models and fitted the following terms in sequential order: block, soil-
299 inoculum treatments and community composition. We did not include plant species
300 richness in this analysis, because it would only have contrasted the three-species
301 mixture with the mean of the three two-species mixtures. However, we also tested
302 whether the overall mean of the NE, CE and SE were different from zero, listing the
303 significance of the intercept in the corresponding analysis of variance (ANOVA) table
304 (Table 2).
305
306 *Effects of community niche on community productivity*
307 In unsterilized soil supplied with a mixture of N forms, where plant species
308 richness was expected to significantly affect community productivity, we tested

309 whether community niche (CN) predicted community productivity. To do this, we
310 replaced plant species richness by CN in the general linear model.
311 All statistical analyses were done in R 3.4.2 (R Development Core Team, 2017).
312 **Results**
313 ***Mycorrhizal root colonization***
314 Plants grown in sterilized soil were relatively free of root colonization by
315 mycorrhiza at the end of the experiment (see Table S1), with a mean colonization of
316 8.1% compared with 66.0% in plants grown in unsterilized soil ($P < 0.001$). For plants
317 grown in unsterilized soil, the percentage of mycorrhizal colonization was 73.7% ± 4.8%
318 (mean ± one standard error) for *C. concinna* (AM fungi), 77.3% ± 6.7% for *O.*
319 *glaberrima* (AM fungi) and 47.0% ± 6.0% for *L. litseifolius* (EM fungi).
320
321 ***Biomass of tree species in response to different N forms***
322 The three tree species differed in biomass on different N forms when inoculated
323 with unsterilized soil in the predicted way (significant ID × N × S interaction; Table 1;
324 Fig. 1). Specifically, each species achieved the highest biomass on a different form of
325 N (home contrast of interaction ID × N in Table 1) and this effect was particularly strong
326 when microbes were present in the soil (home × S contrast of interaction ID × N × S in
327 Table 1; Fig. 1a). The residual interaction terms after removing the home contrasts were
328 very small and insignificant (ID × N and ID × N × S terms following the home and
329 home × S contrasts, respectively, in Table1), suggesting that there were no further
330 differences among the three species with respect to the different N forms on which each

species grew best. In contrast, when inoculated with sterilized soil, all tree species grew equally well on the different forms of N (Fig. 1b).

Effects of soil inoculum on plant biodiversity effects and plant species richness–community productivity relationships

In soils supplied with a mix of N forms, the overall means of the NE and CE were significantly positive and the overall mean of the SE was significantly negative (intercept in Table 2). Soil-inoculum treatments had no significant effects on the NE (Fig. 2 a-b), but significantly affected the CE and SE in an opposite way (soil-inoculum treatments in Table 2). When supplied with a mix of N forms, the CE was significantly larger with than without soil microbes (Fig. 2 c-d), whereas the presence of soil microbes caused a more negative SE (Fig. 2 e-f).

In soils supplied with a mix of N forms, plant community productivity linearly increased with increasing plant species richness (SR in Table 3, model (A)). Moreover, the presence of soil microbes significantly increased community productivity across plant species richness as compared to that without soil microbes (S in Table 3, model (A)), but it did not change the overall pattern of the plant species richness–community productivity relationship (S × SR interaction in Table 3, model (A)). Nevertheless, when analyzed separately, there were non-significant and significantly positive relationships between species richness and productivity in soils without or with microbes, respectively (SR in Table 3, model (B) & model (C)). For communities without N addition, their productivity was not significantly related to plant species richness (SR

353 in Table S2).

354

355 C***ommunity niche predicts community productivity***

356 Our intermixed experiment was designed in such a way that species richness
357 should correlate as highly as possible with community niche, which was indeed the case
358 ($r = 0.93$, $P < 0.001$, see Fig. S1). In addition to that, plant species richness significantly
359 affected community productivity in unsterilized soil supplied with a mix of N forms
360 (SR in Table 3, model (C)), the surrogate parameter CN (community niche) had a
361 similarly positive effect on community productivity (Table 4).

362

363 **Discussion**

364 In this study, we selected three tree species associated with different microbial
365 symbionts (one species with EM fungi, one species with AM fungi and the third species
366 with AM fungi and N-fixing bacteria) to examine microbe-mediated N partitioning and
367 its effects on plant diversity–community productivity relationships. We found that in
368 the presence of microbes each tree species grew best on a different form of N. This
369 suggests that these tree species have the potential for N partitioning. Our study provides
370 the first empirical evidence for microbe-mediated resource partitioning among woody
371 species (Reynolds et al., 2003), but given the small number of species tested, we cannot
372 say how general the phenomenon could be. Previous studies focusing on grassland plant
373 species did not find evidence for it (Reynolds et al., 2005; Vogelsang et al. 2006).
374 Consistent with the observed microbe-mediated plant resource partitioning, our mixed-

species plant communities had larger biodiversity complementarity effects on soils supplied with a mix of N forms in the presence of soil microbes as compared to when soil microbes were absent. Under this condition, community niche, representing the potential of the community to extract N from the soil, was also positively related to community productivity. Our results highlight the potential of microbe-mediated N partitioning among plant species to influence plant diversity–community productivity relationships.

Exploitation of different N forms by different tree species

In sterilized soil, the three tree species grew equally well on the three chemical forms of N, i.e. nitrate, ammonium and organic glycine. In the presence of soil microbes, however, the EM host species *L. litseifolius* grew best with glycine, the AM host species *C. concinna* grew best with nitrate and the AM host and N-fixing species *O. glaberrima* did not show a clear growth distinction but compared with the other two species made best use of ammonium (see Fig. 1). As these differential plant growth responses to N treatments existed in unsterilized soil but not in sterilized soil, it is reasonable to speculate that the differential performance of plant species is related to the direct effect of different N forms rather than the potential different soil pH as a side effect of N addition. We note that our experiment cannot distinguish whether the added N forms were taken up directly by plants or as other N forms after microbial transformation (Harrison, Bol, & Bardgett 2007; Harrison, Bol, & Bardgett 2008), but it does provide evidence that addition of different N forms can cause differential performance of tree

species associated with different mycorrhizal types or other soil microbes. This suggest that the three species had the potential to partition N use if the element was available in the different chemical forms, and that this ability depended on the presence of soil microbes, especially mycorrhizal symbionts, which achieved high root colonization in the experiment. Although our plants interacted with entire soil microbial communities, and although the degree of mycorrhizal colonization is not necessarily strongly correlated with mycorrhizal function (Aerts, 2003), it seems most parsimonious to assign the observed effects mainly to mycorrhiza, with other soil biota contributing to a smaller extent.

These results support our hypothesis of microbe-mediated complementary N-uptake niches among plant species, adding to the evidence that distinct root mycorrhizal symbionts are important for plant nutrient acquisition (Phillips, Brzostek, & Midgley 2013; Steidinger, Turner, Corrales, & Dalling 2015). Some studies with tropical tree species found that partitioning of soil P can occur between or even within plant functional groups (i.e. AM fungi) that host different mycorrhizal types (Steidinger et al., 2015; Nasto et al., 2017). In contrast, another study on more fertile soil only provided weak evidence for N partitioning among tropical tree species with different mycorrhizal types (Andersen, Mayor, & Turner 2017). It is possible that the N partitioning we observed is more common on soils with low overall N availability than on N-rich soils.

Microbe mediated N partitioning among tree species underpinning the positive

419 ***complementarity effect***

420 In the intermixed experiment, we found that soil microbes had a significantly
421 positive effect on the CE when supplied with a mix of N forms, whereas these
422 conditions did not lead to an increased NE because the negative SE counteracted the
423 positive CE. This may explain why soil microbes did not significantly affect the plant
424 species richness–community productivity relationship. However, our hypothesis that
425 microbe-mediated resource partitioning among plant species promote complementary
426 N use in mixed species communities is evident in terms of the complementarity effect
427 CE. It should be noted that the increased CE could not be attributed to the N-fixing
428 species (i.e. *O. glaberrima*) and the associated N-fixing bacteria, because the plant
429 species combinations with this species did not show a high CE (see Fig. 2 c).

430 Moreover, we found similarly positive effects on community productivity of tree
431 species richness and community niche in the presence of soil microbes with added N.
432 Therefore, we conclude that under these conditions the positive complementarity
433 effects are likely driven by community niche related to N extraction. This interpretation
434 is consistent with the suggestion that microbe-mediated resource partitioning between
435 tree species allows mixed-species plant communities to better exploit N when this
436 resource is available in different forms. Our study suggests that even though plant
437 species harbouring different mycorrhiza or other soil microbes have the potential to
438 partition N use, the combination of different species will have no growth benefit if N is
439 not available in multiple forms (Table S2). This corresponds to results of other studies,
440 which also found weaker biodiversity effects when available biotope space was

441 experimentally reduced by changing soil depth or complexity of resource environments
442 (Dimitrakopoulos & Schmid, 2004; Jousset, Schmid, Scheu, & Eisenhauer 2011).

443 One previous study found that plants associated with one mycorrhizal type can
444 promote the growth of their neighbors with different root symbionts (Teste, Veneklaas,
445 Dixon, & Lambers 2014). We took that idea a step further and show that tree species
446 with different mycorrhizal types potentially partition N via soil microbes, which can
447 promote complementarity effects in communities. Our results do not exclude the
448 possibility that different plants in a community may further trade nutrients through
449 common hyphal networks and this may further affect community productivity (Simard
450 & Durall, 2004; Walder et al., 2012; Klein, Siegwolf, & Körner 2016). However, such
451 exchange requires common hyphal networks (Simard & Durall, 2004) and thus should
452 not occur between species associating with different types of mycorrhizal fungi. Thus,
453 potential hyphal links should not alter our main conclusion that N partitioning among
454 tree species promoted complementarity effects of mixed-species communities.

455

456 ***Implications for species coexistence and ecosystem functioning***

457 If and where the microbe-mediated soil nutrient partitioning does occur in forest,
458 it may not only increase resource extraction by mixed-species plant communities but
459 also facilitate plant species coexistence in complex resource environments. Although
460 plant–mycorrhiza interactions have been repeatedly shown to affect the coexistence of
461 different plant species and biodiversity–productivity relationships (van der Heijden et
462 al., 1998; Klironomos, Mccune, Hart, & Neville 2000; Maherali & Klironomos, 2007;

van der Heijden, Bardgett, & van Straalen 2008; Wagg et al., 2015; Bennett et al., 2017; Jiang et al., 2017; Luo, De Deyn, Jiang, & Yu 2017; Teste et al., 2017), our case study reveals the role of soil microbes in plant-N partitioning. Our species pool contained only three tree species, therefore we cannot extrapolate the results to a broader set of mature canopy trees in natural forest. However, we at least provide a proof of principle for the mediation of N partitioning by soil microbes, which furthermore may promote plant species coexistence by minimizing interspecific competition.

Our study also provides a proof of principle that microbe-mediated plant N partitioning can enhance the CE component of the net biodiversity effect (NE), even though at the expense of a decreased SE component. Thus, our study establishes a mechanistic link between complementary resource use and the positive plant diversity–community productivity relationship (Turnbull, Levine, Loreau, & Hector 2013). Disadvantages of our study are the relatively short duration of 14 months and the use of tree seedlings rather than larger plant individuals. The complementarity among plant species is likely to increase over time, and the effects of soil microbes on plants may have a time lag (Cardinale et al., 2007; Fargione et al., 2007; Eisenhauer, Reich, & Scheu 2012; Reich et al., 2012). It is therefore conceivable that the observed microbe-mediated N partitioning among tree species, as well as the positive complementarity effect, would be even larger in longer-term experiments.

In conclusion, the complementing results of our two experiments provide evidence that plant resource partitioning mediated by soil microbes may be an underlying mechanism for efficient resource extraction by species-rich plant

485 communities in environments with complex forms of elemental nutrients. Our study
486 expands previous concepts of complementary resource use by showing the intimate
487 connections between plant–microbe associations, plant niche partitioning and
488 ecosystem functioning. Future studies applying our proofs of concepts to natural forest
489 ecosystems with higher species diversity, longer time scales and *in situ* experimentation
490 may provide further mechanistic insight into species coexistence and biodiversity–
491 ecosystem functioning relationships.
492
493 **Author's contributions**
494 SL designed the experiment with advices from SY, and conducted the experiment. SL
495 and BS analyzed the data. SL wrote the first draft of the manuscript, and all authors
496 discussed the results and contributed substantially to revisions.
497
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Data Accessibility

The data supporting the results are archived in Dryad: https://doi.org/10.5061/dryad.96r47g0 (Luo, Schmid, De Deyn, & Yu 2018)

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744
745 **Supporting Information**
746 Details of Supporting Information are provided below.
747 **Table S1.** Root mycorrhizal colonization of each tree species.
748 **Table S2.** Relationship between plant species richness and community productivity in

749 unsterilized soil without N addition

750 **Fig. S1.** Relationship between plant species richness and community niche.

751 **Appendix S1.** Supporting analyses

752 **Table 1.** Summary of general linear model results showing the effects of plant species identity, N treatments (ammonium, nitrate or glycine
753 addition), soil-inoculum treatments (sterilized vs. unsterilized inoculum) and their interactions on the total above- and belowground biomass of
754 plant individuals. The preferred N form of each species was considered as its "home" N form.

		Plant biomass			
Source of variation		df	%SS	*F*	*P*
Plant species identity (ID)		2	13.02	20.76	**＜0.001**
N treatments (N)		2	0.65	1.03	0.360
Soil inoculum treatments (S)		1	0.02	0.08	0.781
ID × N		4	3.59	2.87	**0.024**
	Home	*1*	*3.34*	*10.69*	***0.001***
	ID × N	*3*	*0.25*	*0.26*	*0.853*
ID × S		2	0.63	0.99	0.373
N × S		2	2.37	3.78	**0.024**
ID × N × S		4	3.20	2.55	**0.040**
	Home × S	*1*	*2.82*	*9.00*	***0.003***
	ID × N × S	*3*	*0.38*	*0.40*	*0.755*
Residuals		244	76.53		

755 Key to abbreviations: df, degree of freedom; %SS, percentage of the total sum of squares explained by each term; the numbers in bold indicate
756 significant effects; in italics are the decompositions of previous terms (namely ID × N and ID × N × S) and corresponding statistical values.
757

Table 2. Results of general linear models presenting the effects of block, soil-inoculum treatments (sterilized vs. unsterilized inoculum) and community composition (different combination of species) on NE, CE and SE in soil supplied with a mix of N forms. The interaction term soil-inoculum treatments × community composition was not significant and therefore omitted from the analysis.

		NE			CE			SE		
Source of variation	df	SS %	*F*	*P*	SS %	*F*	*P*	SS %	*F*	*P*
Intercept	1	16.48	14.98	**<0.001**	18.47	18.78	**<0.001**	11.00	10.88	**0.002**
Block	9	17.72	1.79	0.090	18.26	2.06	**0.048**	21.42	2.35	**0.025**
Soil-inoculum treatments	1	1.80	1.64	0.206	4.17	4.24	**0.044**	4.18	4.13	**0.047**
Community composition	3	1.29	0.39	0.759	3.03	1.03	0.387	5.73	1.89	0.144
Residuals	57	62.71			56.06			57.67		

Key to abbreviations: df, degree of freedom; %SS, percentage of the total sum of squares explained by each variable; the numbers in bold indicate significant effects.

763 **Table 3.** Results of general linear model analyzing the relationships between plant
764 species richness and community productivity (the total of plant above- and
765 belowground biomass) in soils supplied with a mix of N forms but with different soil-
766 inoculum treatments (sterilized vs. unsterilized inoculum). Overall analysis (Model A)
767 followed by separate analyses for soils with sterilized (Model B) and unsterilized
768 inoculum (Model C).

Model A: overall model				
	Community productivity			
Source of variation	df	% SS	*F*	*P*
Block	9	7.20	1.82	0.072
Soil-inoculum treatments (S)	1	7.46	16.96	**<0.001**
Plant species richness (SR)	1	2.51	5.70	**0.019**
S × SR	1	0.34	0.77	0.381
Community composition	5	30.11	13.68	**<0.001**
Residuals	119	52.38		

Model B: sterilized inoculum				
	Community productivity			
Source of variation	df	% SS	*F*	*P*
Block	9	12.44	1.54	0.159
Plant species richness (SR)	1	1.40	1.55	0.217
Community composition	5	40.40	9.00	**<0.001**
Residuals	51	45.77		

Model C: unsterilized inoculum				
	Community productivity			
Source of variation	df	% SS	*F*	*P*
Block	9	21.76	2.80	0.009
Plant species richness (SR)	1	3.78	4.38	**0.041**
Community composition	5	27.78	6.43	**<0.001**
Residuals	54	46.67		

769 Key to abbreviations: df, degree of freedom; %SS, percentage of the total sum of
770 squares explained by each variable; the numbers in bold indicate significant effects.

771

772 **Table 4.** Results of general linear model analysis presenting the effect of community
773 niche (CN) on community productivity (the total of plant above- and belowground
774 biomass) in unsterilized soil supplied with a mix of N forms.

	Community productivity			
Source of variation	df	% SS	*F*	*P*
Block	9	21.76	2.80	**0.009**
Community niche	1	3.57	4.13	**0.047**
Community composition	5	28.00	6.48	**<0.001**
Residuals	54	46.67		

775 Key to abbreviations: df, degree of freedom; %SS, percentage of the total sum of
776 squares explained by each variable; the numbers in bold indicate significant effects.
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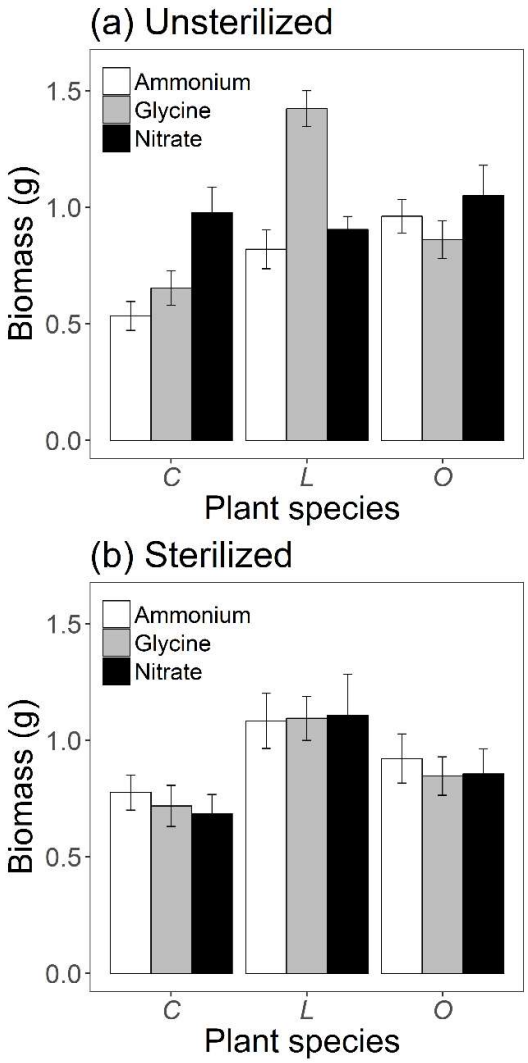


791

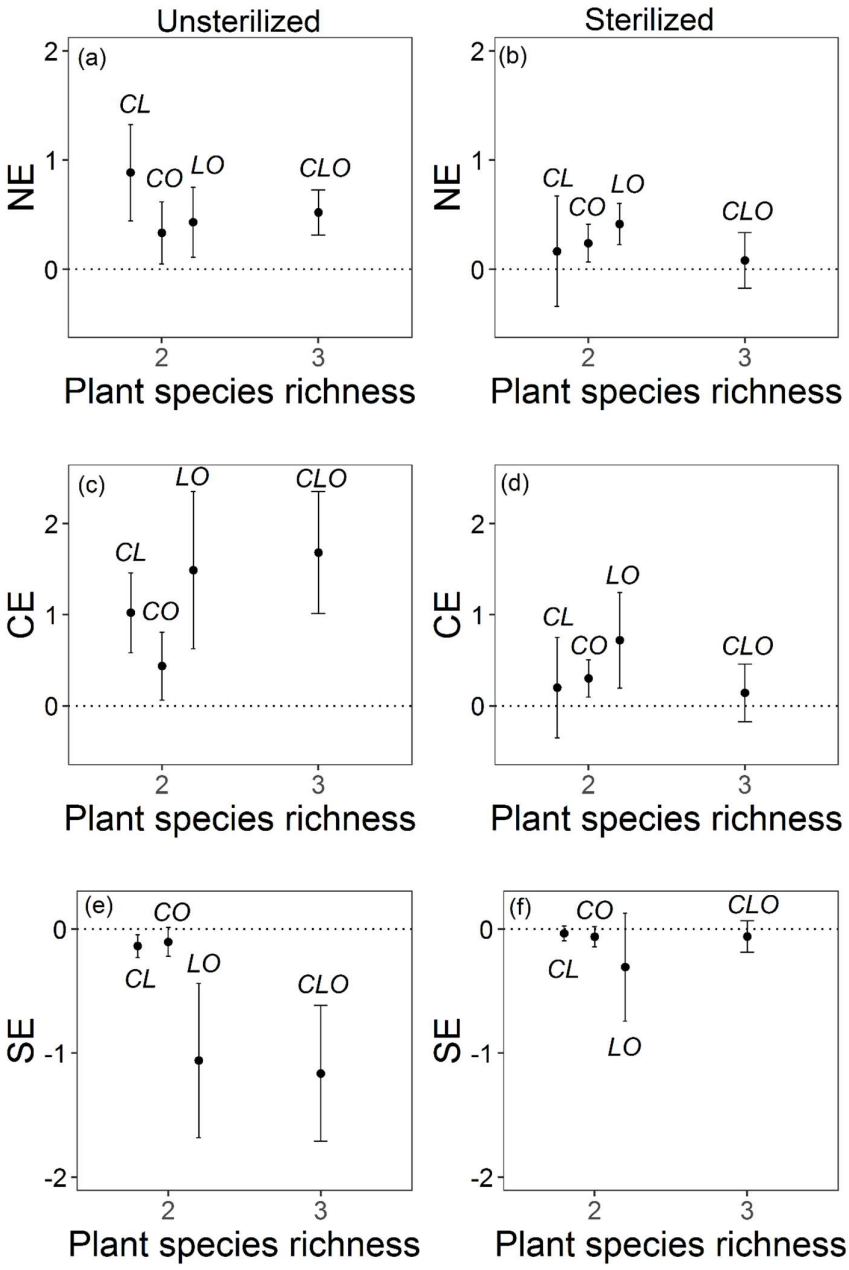
792 **Fig. 1.** Mean biomass (the total of above- and belowground biomass dry weight) of

793 plant individuals in soils supplied with different N forms (ammonium, glycine or nitrate)

794 but with either unsterilized (a) or sterilized inoculum (b). Data shown are means ± SEM

795 (N = 15). Species code: *C*, *Cryptocarya concinna*; *L*, *Lithocarpus litseifolius*; *O*,

796 *Ormosia glaberrima*.



797

798 **Fig. 2.** Net effect (NE; a-b), complementarity effect (CE; c-d) and selection effect (CE;

799 e-f) of different plant species composition (2 or 3 species) in soils supplied with a mix

800 of N forms but with either unsterilized (left panel) or sterilized inoculum (right panels).

801 Data shown are means ± SEM (N = 10). Species code: *C*, *Cryptocarya concinna*; *L*,

802 *Lithocarpus litseifolius*; *O*, *Ormosia glaberrima*.

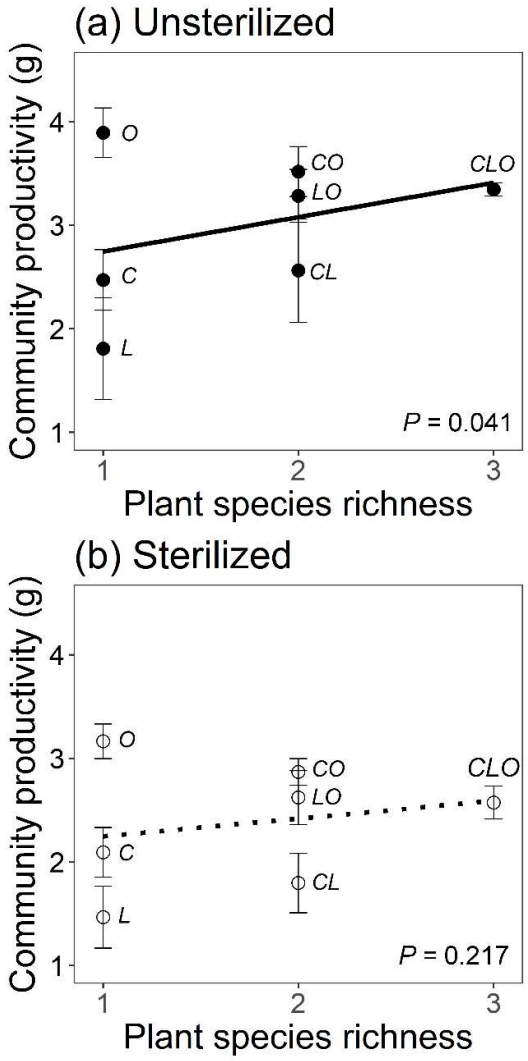


Fig. 3. Relationships between plant species richness and community productivity (the total of plant above- and belowground biomass) in soils supplied with a mix of N forms but with either unsterilized (a) or sterilized inoculum (b). Solid line indicates significant ($P < 0.05$) and dotted line indicates insignificant ($P > 0.2$) relationship between plant species richness and community productivity (see Table 3). The mean (± SE) is shown for each community composition under different treatments ($N = 10$). Species code: *C*, *Cryptocarya concinna*; *L*, *Lithocarpus litseifolius*; *O*, *Ormosia glaberrima.*

Functional Ecology

Supporting Information

Appendix S1. Supporting analyses

Soil microbes promote complementarity effects among co-existing trees through soil nitrogen partitioning

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Table S1. Percentage of root mycorrhizal colonization of each tree species under unsterilized and sterilized soils.

Species	Unsterilized	Sterilized
Cryptocarya concinna	73.7 % ± 4.8%	9.3% ± 2.3%
Lithocarpus litseifolius	47.0 % ± 6.0%	5.8% ± 1.1%
Ormosia glaberrima	77.3 % ± 6.7%	9.1% ± 1.1%

Table S2. Result of general linear model analyzing the relationships between plant species richness and community productivity (the total of plant above- and belowground biomass) in unsterilized soil without N addition.

	Community productivity			
Source of variation	df	% SS	*F*	*P*
Block	9	29.03	3.40	**0.002**
Plant species richness (SR)	1	0.02	0.03	0.876
Community composition	5	21.63	4.56	**0.002**
Residuals	52	49.32		

Key to abbreviations: df, degree of freedom; %SS, percentage of the total sum of squares explained by each variable; the numbers in bold indicate significant effects.

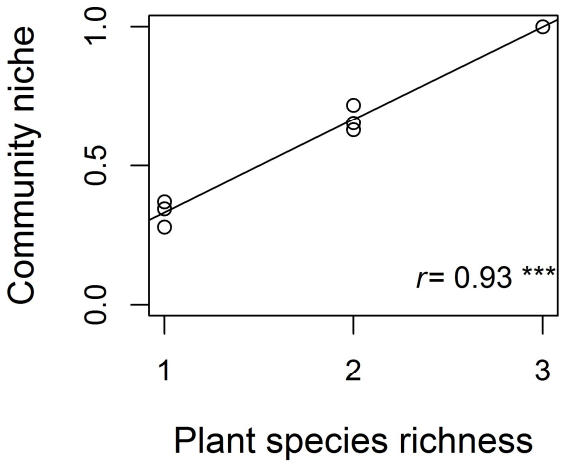

Fig. S1. Relationship between plant species richness and community niche. Significance level for correlation coefficient ≠ 0: ***, $P < 0.001$