ACCEPTED VERSION

M. Hoogmoed, S.C. Cunningham, P. Baker, J. Beringer, T.R. Cavagnaro
N-fixing trees in restoration plantings: effects on nitrogen supply and soil microbial communities
Soil Biology and Biochemistry, 2014; 77:203-212

Final publication at http://dx.doi.org/10.1016/j.soilbio.2014.06.008

PERMISSIONS

https://www.elsevier.com/about/policies/sharing

Accepted Manuscript

Authors can share their accepted manuscript:

<mark>24 Month Embargo</mark>

After the embargo period

- via non-commercial hosting platforms such as their institutional repository
- via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license – this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy
- not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article

18 August 2021

---

http://hdl.handle.net/2440/86222

1   **Title:** N-fixing trees in restoration plantings: effects on nitrogen supply and soil microbial

2   communities.

3

4   **Authors:**

5   M. Hoogmoed[a], marianne.hoogmoed@monash.edu

6   S.C. Cunningham[a, b, c], shaun.cunningham@deakin.edu.au

7   P. Baker[a, d], patrick.baker@unimelb.edu.au

8   J. Beringer[e, f], jason.beringer@monash.edu

9   T. Cavagnaro[a, g], timothy.cavagnaro@adelaide.edu.au

10

11   **Affiliations:**

12   [a] School of Biological Sciences, Monash University, Victoria 3800, Australia.

13   [b] School of Life and Environmental Sciences, Burwood Campus, Deakin University, Victoria 3125,

14   Australia.

15   [c] Institute for Applied Ecology, University of Canberra, ACT 2601, Australia

16   [d] Department of Forest and Ecosystem Science, Burnley Campus, Melbourne University, Victoria

17   3121, Australia.

18   [e] School of Geography and Environmental Science, Monash University, Victoria 3800, Australia.

19   [f] School of Earth and Environment, University of Western Australia, Crawley 6009, WA, Australia

20   [g] School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1 Glen Osmond,
21   South Australia 5064, Australia.

22

23   **Corresponding author:**
24   Tim Tim Cavagnaro
25   University of Adelaide, School of Agriculture, Food and Wine
26   Waite Campus
27   PMB1 Glen Osmond
28   South Australia 5064, Australia.
29   Tel.: +61 8 8313 2770

30

Abstract

Mixed-species restoration tree plantings are being established increasingly, contributing to mitigate climate change and restore ecosystems. Including nitrogen (N)-fixing tree species may increase carbon (C) sequestration in mixed-species plantings, as these species may substantially increase soil C beneath them. We need to better understand the role of N-fixers in mixed-species plantings to potentially maximize soil C sequestration in these systems. Here, we present a field-based study that asked two specific questions related to the inclusion of N-fixing trees in a mixed-species planting: 1) Do non-N-fixing trees have access to N derived from fixation of atmospheric N_2 by neighbouring N-fixing trees? 2) Do soil microbial communities differ under N-fixing trees and non-N-fixing trees in a mixed-species restoration planting? We sampled leaves from the crowns, and litter and soils beneath the crowns of two N-fixing and two non-N-fixing tree species that dominated the planting. Using the ^{15}N natural abundance method, we found indications that fixed atmospheric N was utilized by the non-N-fixing trees, most likely through tight root connections or organic forms of N from the litter layer, rather than through the decomposition of N-fixers litter. While the two N-fixing tree species that were studied appeared to fix atmospheric N, they were substantially different in terms of C and N addition to the soil, as well as microbial community composition beneath them. This shows that the effect of N-fixing tree species on soil carbon sequestration is species-specific, cannot be generalized and requires planting trails to determine if there will be benefits to carbon sequestration.

1. Introduction

Afforestation of agricultural land may contribute to carbon sequestration, potentially mitigating climate change, and restoring of native ecosystems (Guo and Gifford, 2002; Hoogmoed et al., 2012; Paul et al., 2002). Single-species tree plantations for wood production are among the most common afforestation systems (Chazdon, 2008; Paul et al., 2002), although restoration plantings, which contain a mixture of native tree species that are not harvested, are becoming more widely planted (Cunningham et al., 2012). This is because in addition to their potential capacity to store carbon, both above- and below-ground, they provide a range of additional ecological benefits (Harrison et al., 2000), including increased habitat for native flora and fauna (Munro et al., 2009) and ecological stability (e.g. higher resilience to insect pests, Knoke et al., 2008), and nutrient interception when planted as buffer strips adjacent to waterways (Burger et al., 2010; Fennessy and Cronk, 1997).

A fundamental question in establishing mixed-species restoration plantings is which species to plant. One consideration in selecting tree species is whether individual species possess desirable traits. For example, nitrogen-fixing trees can directly fix atmospheric nitrogen (N) to support partly or totally their own growth, giving them an advantage over non-N-fixing tree species, especially in N limited systems (Galiana et al., 1998). Consequently, higher levels of soil C under N-fixing trees have been attributed to higher growth rates of N-fixing trees and subsequent higher C inputs into the soil via litter and root exudates (e.g. Resh et al., 2002; Wang et al., 2010). Including N-fixing tree species in mixed-species restoration plantings may increase and accelerate the carbon sequestration potential of the ecosystem (Kaye et al., 2000). In addition to increasing soil N (Kaye et al., 2000), heightened N levels may reduce lignin decomposition (e.g. Berg and Matzner, 1997; Carreiro et al., 2000), further slowing organic matter decomposition and increasing C sequestration (Prescott, 2010).

In mixed-species plantings, N-fixing trees can also facilitate the growth of non-N-fixers. The non-N-fixers may benefit from lowered competition for the available soil N, or they may be able to access the fixed atmospheric N pool (Forrester et al., 2006) after decomposition of the N-fixers litter (van

83   Kessel et al., 1994), through root exudates, or via interconnected mycorrhizal networks between the

84   trees (He et al., 2003). This facilitative effect of N-fixers on non-N-fixers is important for net primary

85   production, as well as community development (Siddique et al., 2008) and successional processes

86   (Chapin et al., 1994; Vitousek and Walker, 1989). Consequently, the inclusion of N-fixers in mixed

87   species woody plants may have an important impact upon N dynamics in these systems.

88   The stand-scale consequences of N_2-fixation on soil C sequestration are ultimately driven by the

89   effects of N on soil processes. This may include impacts on soil microbial communities, which play a

90   key role in organic matter decomposition (Wardle, 2002). This process is governed by complex

91   interactions among factors such as litter quantity and quality (nutrient content and chemical

92   structure), soil microbial community composition and several biotic and abiotic factors (e.g. Prescott,

93   2010). Soil microbial communities are often found to differ among tree species (Priha et al., 2001),

94   presumably, due to differences in litter quality and quantity (Bauhus et al., 1998; Hobbie, 1992;

95   Schweiter et al., 2012). Higher amounts of N in litter and soil under N-fixing trees are likely to have a

96   major effect on the soil microbial community beneath these trees (Allison et al., 2006). For example,

97   higher available nitrogen or a lower C:N ratio under N-fixers may favour bacterial over fungal

98   decomposers (Fierer et al., 2009; Harrison and Bardgett, 2010). Bacteria are generally less adapted

99   to decompose recalcitrant litter as fungi (Henriksen and Breland, 1999; van der Heiden et al., 2008).

100   Therefore, increased N levels under N-fixing trees may shift the microbial community towards

101   bacterial dominance, slowing the rate of decomposition of organic matter and increasing the rate of

102   soil C sequestration. In contrast, fungal biomass is more recalcitrant and fungi have a higher C

103   assimilation efficiency compared with bacteria, therefore a shift towards more bacteria could also

104   result in a reduction of soil C sequestration (Bailey et al., 2002b).

105   If the potential for N-fixers to increase soil C sequestration in mixed-species afforestation

106   plantings is to be maximized, we need to better understand the role of N-fixers in these plantings.

107   An extensive literature exists on interactions between N-fixing and non-N-fixing trees (e.g., Bouillet

108 et al., 2013; Forrester, 2014), albeit predominantly in relation to tree growth and wood production

109 (e.g. Binkley et al., 2003; Parrotta, 1999) but also soil C sequestration (e.g. Kaye et al., 2000) or

110 nutrient cycling (e.g. Khanna, 1997). However, there is a lack of consensus about how N-fixers and

111 non-N-fixers interact and what drives differences among studies. Further, little is known about the

112 impact of N-fixers on soil microbial communities in mixed-species plantings. Here, we present the

113 results of a field-based study in which we investigated two important aspects of restoration

114 plantings including both N-fixing and non-N-fixing tree species: 1) the pathways that fixed

115 atmospheric N takes within the stand and 2) the effect of N-fixers on the soil microbial community.

116 We asked two specific questions:

117   1. Do non-N-fixing trees have access to N derived from the fixation of atmospheric N_2 by

118   neighbouring N-fixing trees, in the early development of a tree planting?

119   2. Do changes in the N dynamics associated with N-fixing trees, result in changes in soil

120   microbial communities in a mixed-species restoration planting?

121   To address these questions, we focused on a young (14 yr) mixed-species planting in

122 southeastern Australia.

123

2. Materials and methods

2.1. Site description

A field study was conducted in November 2011, in a mixed-species restoration planting along Castle Creek near Euroa (36'86°S, 145'58°E) in northern Victoria, south-eastern Australia. The region has a temperate climate with an mean annual rainfall of 650 mm, ranging from 30 to 80 mm month^{-1}, monthly maximum temperatures between 12.3 and 29.7 °C and monthly minimum temperatures between 4.1 and 15.3 °C (1981 – 2010, Australian Bureau of Meteorology, 2011). The site was previously a pasture that was replanted in 1997 with a mixture of tubestock seedlings of N-fixing and non-N-fixing trees. The N-fixers were *Acacia dealbata* Link*., A. implexa* Benth*, A. melanoxylon* R. Br., and the non-N-fixers were *Eucalyptus camaldulensis* Dehnh. *, E. polyanthemos* Schauer*, E. macrorhyncha* F. Muell*, E. macrocarpa* Maiden and various shrubs. Tree density was ca 700 trees ha^{-1} and basal area was 13.9 m^2 ha^{-1} at the time of sampling. Soil was a Chromosol loam, classified as Pb1 according to the Australian Soil Classification System (ABARES, 2004), with a mean pH of 5.1.

2.3. Sampling

The two dominant N-fixing tree species, *Acacia dealbata* and *A. implexa*, and the two dominant non-fixing tree species, *Eucalyptus camaldulensis* and *E. polyanthemos*, were selected to study N cycling and soil microbial communities in the restoration planting. Ten trees of each species were randomly selected within a 1 ha plot, and sampled for soil, litter and fresh leaves. The selected trees covered the range of DBH (diameter at breast height) of each species within the planting: *A. dealbata* (14 – 23 cm), *A. implexa* (7 – 20 cm) *E. camaldulensis* (9 – 25 cm) and *E. polyanthemos* (15 – 35 cm). Soil was sampled from two depth layers (0-10 and 10-20 cm) under the crown of each of the selected trees, on average 50 cm, and never more than 1 m away from the base of the stem. In the 0-10 cm layer, four subsamples (*ca* 100 g) were collected around the stem in different directions and then

148   bulked to make one composite sample. In the 10-20 cm layer, two samples (*ca* 200 g) were collected

149   to make one composite sample. Given limited differences $\delta^{15}N$ among the tree types (see results),

150   we collected additional soil samples from a large patch of non-N-fixing trees to provide a reference

151   value for $\delta^{15}N$ in soil with negligible influence of N-fixing trees. In June 2013, five soil samples were

152   collected in the patch from the 0-10 cm layer, which was ca 10 m away from the nearest N-fixing

153   tree. All soil, from both sampling campaigns was stored immediately at 4 °C for 2 days until further

154   processing in the laboratory. Soil bulk density samples were taken at both depth layers, under six of

155   the N-fixers and six of the non-fixers, making sure that trees were spread across the whole sampling

156   area, following Minoshima et al. (2007).

157   To assess the presence of fixed atmospheric N in litter and fresh leaves, standing litter was

158   collected from within a randomly placed 20 cm x 20 cm quadrat underneath the crown of each tree,

159   within 1 m of the base of the stem. Representative samples of fully-expanded leaves were collected

160   from each tree from four locations in the crown: at two randomly selected sides of the tree and at

161   two heights (from the lowest branches and up to 10 m) using pruning shears on an extension pole.

162

163   *2.4. Sample processing*

164   Soil samples were passed through a 2 mm sieve. A subsample was frozen immediately at -20 °C for

165   phospholipid fatty acid (PLFA) analysis (see below). Soil moisture was determined by drying a

166   subsample of *ca* 10 g moist soil samples at 105 °C for 48 h.

167   All remaining soil was air-dried, and a subsample was ground to a fine powder using a mill

168   and analysed for total C and total N, and the $\delta^{15}N$ value and pH. Values of $\delta^{15}N$ are defined as the

169   ratio between ^{15}N and ^{14}N isotopes in the sample, and are used to trace the fate of N in ecosystems

170   (Robinson, 2001). Elemental and isotope analysis was done using dry combustion in an ANCA GSL 2

171   elemental analyzer (Sercon Ltd., UK), coupled to a Hydra 20-22 isotope ratio mass-spectrometer

172 (Sercon Ltd., UK). The precision for ^{15}N is 0.1‰. Please note: total C and total N means per tree type

173 (i.e. the species grouped as N-fixing and non-N-fixing) were published previously in Hoogmoed *et al*.

174 (2014). The pH of the air dried soil was measured in a 1:5 soil water slurry using a TPS WP-81 pH,

175 TDS,Temperature & Conductivity Meter (EnviroEquip Biolab, Australia).

176   Bulk density samples were dried at 105 °C for 48 h. Stones were retained to estimate stone

177 volume in each sample using displacement of water in a measuring cylinder. Bulk density was

178 calculated by dividing the oven-dried soil mass by the steel cylinder volume less the stone volume.

179   To compare soil microbial communities among the tree species, phospholipid fatty acid

180 (PLFA) analysis following the procedures of Bossio et al. (1998) with slight modifications (Mosse et al.,

181 2012). PLFA analysis was performed on the 0-10 cm soil layer only, as we assume that microbial

182 activity is most predominant in this soil layer (e.g., Fierer et al., 2003; Hossain et al., 1995).

183   Briefly, PLFAs were extracted from 4 g freeze dried, grinded soil samples, using a solvent

184 containing citrate buffer (0.15 M, pH 4.0), chloroform and methanol, followed by transesterification

185 of the polar lipid fraction containing the phospholipids. Separation of PLFAs was done using gas

186 chromatography (30 m (5%-phenyl)-methylpolysiloxane column (Varian CP 3800)). Peaks were

187 identified and quantified by comparing with Supelco Bacterial Acid Methyl Ester (BAME) standard

188 mix (product number 47080-U, Supelco, USA). Nomenclature of PLFAs followed that of Frostegård

189 and Bååth (1996).

190   Litter and fresh leaves were oven dried at 60 °C for 48 hr. Identifiable leaves of the tree

191 species under which the sample was taken were removed from the bulk sample for analysis of the

192 species-specific leaf litter. The remaining litter and the species-specific leaf-litter were then ground

193 to powder with a biomass grinder (IKA, Malaysia). Small subsamples (*ca.* 5 mg) of the species-specific

194 leaf-litter were used for total C, total N and δ^{15}N analysis. The remaining species-specific leaf-litter

195 was returned to the main litter sample and analysed to obtain total C, total N and δ^{15}N for the whole

196 litter sample. Fresh leaves were also ground and analysed for C, N and δ^{15}N.

197

198 *2.5. Statistical analysis*

199   All statistical analyses were performed using the statistical software R (version 3.0.0., R Core

200 Team, 2013). To trace the fate of atmospheric N (δ^{15}N), total C, total N and C:N ratio in the

201 restoration planting, the effects of *tree type* (*Acacia dealbata* and *A. implexa* pooled as 'N-fixers' and

202 *Eucalyptus camaldulensis* and *E. polyanthemos* pooled as 'non-N-fixers') was analysed by a nested-

203 analysis of variance (ANOVA), with *tree species* nested in *tree type*, for each sample type separately.

204 Paired t-tests were performed to test differences among species. Differences in δ^{15}N, total C, total N

205 and C:N ratio among sample types within a tree species were analysed by one-way-ANOVA. For the

206 one-way-ANOVA of total C and total N content of the sample types, the analysis only included leaves,

207 species-specific litter and litter, because of different measurement units used for the soil C and N

208 stocks (t ha^{-1}) compared with the leaves and litter (%). For δ^{15}N and C:N ratio, all sample types were

209 compared. Results for total C and total N content in the 0-10 cm and 10-20 cm soil layer have been

210 reported previously in Hoogmoed et al. (2014) but are included in the results section for

211 completeness.

212   Non-metric multidimensional scaling (NMDS) was performed using the metaMDS function

213 within the *vegan* package (Oksanen et al., 2013) to explore dissimilarities in PLFA communities

214 among tree types and tree species. PLFAs with a concentration of less than 0.1 mg L^{-1} were

215 considered absent. Only PLFAs detected in more than 4% of the samples were included in the

216 analysis and some of the PLFAs were excluded when also found in the blank samples. In total, 16

217 PLFAs were used in analysis. The PLFA data were first normalized by sample mass and then range

218 standardized, scaling values between 0 and 1. The dissimilarity in PLFA communities among the

219 samples was estimated using the Bray–Curtis metric (Bray and Curtis, 1957). Analysis of dissimilarity

220 was performed using the *adonis* function within the *vegan* package, to test whether PLFAs were

221 significantly dissimilar between N-fixers and non-N-fixers, and among the individual tree species. To

222   determine which environmental variables explained most of the variation in microbial community

223   composition beneath trees, we used the *envfit* function in the *vegan* package. Vectors of variables

224   that were significantly correlated ($P < 0.05$) and explained more than 50% of the variation ($R^2 > 0.50$)

225   in the microbial communities were plotted on the NMDS ordination (Figure 1). The following

226   environmental variables were included in the analysis from the 0-10 cm soil layer: PMN, NH_4^+, NO_3^-

227   and total mineral N ($NH_4^+ + NO_3^-$, as reported in Hoogmoed et al. (2014), Table S2, site R1), soil

228   moisture, total N, total C, C:N ratio, pH and total amount of PLFA.

229   The fungal-to-bacterial ratio (F:B ratio) was calculated using the PLFA marker 18:2ω6,9c, as

230   an indicator of fungal biomass, and the sum of PLFA markers i15:0, a15:0, i16:0, i17:0, 17:0cy, 17:0

231   and 19:0cy as an indicator of total bacterial biomass (Frostegård and Bååth, 1996). Differences in F:B

232   ratio, total PLFA and individual PLFAs were tested using the same nested-ANOVA design as

233   described above. Pearson correlation analysis was used to test relationships between F:B ratio and

234   C:N ratio, total C and total N, and between total fungal PLFA and C:N ratio, total N and total C, in the

235   0-10 cm soil layer.

236 **3. Results**

237 *3.1. Nitrogen cycling*

238 There were no significant differences ($P < 0.05$) in the δ^{15}N values between tree types (N-fixers and

239 non-N-fixers) for any of the sample types (leaves, species-specific litter, litter, 0-10 cm soil layer and

240 10-20 cm soil layer, Table 1). However, there were significant differences in δ^{15}N value of the soil

241 (both 0-10 and 10-20 cm soil layers) among tree species within tree type ($P < 0.01$, Table 1). Soil

242 underneath *A. dealbata* had a significantly higher δ^{15}N value compared with the other tree species in

243 the 0-10 cm soil layer ($P \leq 0.03$). In the 10-20 cm soil layer, δ^{15}N under *A. implexa* was significantly

244 higher compared with *A. dealbata* and *E. camaldulensis* ($P \leq 0.02$).

245 The δ^{15}N values of the different s*ample types* were significantly different in all tree species (P

246 < 0.01, Table 1). *Acacia dealbata, A. implexa* and *E. camaldulensis* showed no significant differences

247 among leaves, species-specific litter and litter, but these were significantly lower compared with the

248 δ^{15}N value of the soil, in both depths ($P \leq 0.01$). *Eucalyptus polyanthemos* showed a significantly

249 lower δ^{15}N value in the species-specific litter compared with the leaves and litter ($P \leq 0.01$).

250 The δ^{15}N value of soil that was sampled at a later time, as far away as possible from any N-

251 fixing trees, was higher (6.1 ± 0.32) than under the N-fixing (4.97 ± 0.22) and non-N-fixing ($4.75 \pm$

252 0.12) trees that were grown closer together. As these samples were collected at different times, the

253 difference between the values should be treated as an indication of relative difference rather than

254 an absolute difference.

255 Total N content was higher in leaves and litter of the N-fixing trees compared with the non-

256 N-fixing trees. However, a species within tree-type effect was also found for leaves, litter and both

257 soil layers (Table 3). In leaves and species-specific litter, total N content was significantly higher in *E.*

258 *camaldulensis* compared with *E. polyanthemos* ($P < 0.01$). In the soil layers, total N content was

259 significantly higher under *A. dealbata* compared with *A. implexa* ($P < 0.01$).

260   Comparing total N concentration among leaves, species-specific litter and litter (soil was not

261   compared as the units differed, see Materials and Methods) for each tree species, showed

262   significant differences among sample types for all tree species ($P < 0.01$). Leave N content was

263   significantly higher compared with litter N content, for all species ($P ≤ 0.03$). Species-specific litter N

264   content was significantly lower compared with leaves for *A. dealbata*, *A. implexa* and *E.*

265   *polyanthemos* ($P ≤ 0.01$), and significantly lower than litter underneath *E. polyanthemos* ($P < 0.01$).

266

267   *3.2. Carbon*

268   There were no differences in total C content between N-fixers and non-N-fixers for any of

269   the sample types (Table 3). However, species within tree-type effects were found for species-specific

270   litter, and both soil layers. Carbon content was higher in samples of *A. dealbata* compared with *A.*

271   *implexa*. However post-hoc testing revealed that for species-specific litter, this difference was only

272   marginal compared with *A. implexa* ($P = 0.051$). There was no difference in C content among samples

273   of the non-N-fixing species.

274   There were no significant differences in C content between leaves and species-specific litter,

275   for any of the tree species, but litter had a significantly lower C content compared with leaves for all

276   species ($P ≤ 0.05$) except for *E. polyanthemos* ($P = 0.17$).

277

278   *3.3. C:N ratio*

279   The C:N ratio was significantly lower in litter under N-fixing trees compared with the non-N-fixing

280   trees ($P ≤ 0.02$, Table 3). A species within tree type effect was found for all sample types except litter

281   ($P = 0.73$, Table 3). The C:N ratio was significantly higher in *E. polyanthemos* compared with *E.*

282   *camaldulensis* in all sample types ($P ≤ 0.04$) expect litter ($P = 0.64$). Between the N-fixing trees, the

283   C:N ratio was only significantly higher in the 0-10 cm soil layer under *A. implexa* compared with *A.*

284   *dealbata* (P < 0.01, Table 4).

285   There were several differences the C:N ratio among sample types within each tree species.

286   Leaves, species-specific litter, litter and soil had significantly different C:N ratios for *A. dealbata* and

287   *A. implexa*. Soil under both non-N-fixing species had a significantly lower C:N ratio compared with

288   the rest of the sample types ($P < 0.01$). The C:N ratio in litter of *E. camaldulensis* was significantly

289   higher compared with leaves ($P < 0.01$), but litter and leaves did not differ significantly from species-

290   specific litter ($P = 0.96$). For *E. polyanthemos*, the C:N ratio of leaves and litter did not differ, but it

291   was significantly higher in species-specific litter.

292

293   *3.4. Soil microbial community*

294   Soil microbial community composition, measured using PLFAs, did not differ significantly under N-

295   fixing and non-N-fixing trees ($P = 0.08$, Figure 1). Among all soil samples irrespective of tree type,

296   total PLFA concentration explained most of the variation in microbial communities ($R^2 = 0.84$, $P <$

297   0.01) followed by total C and total N content of the soil ($P < 0.01$, $R^2 = 0.54$ and $P < 0.01$, $R^2 = 0.51$

298   respectively). Comparing individual tree species, soil microbial communities under *A. dealbata* were

299   significantly different compared with the other three tree species ($P \leq 0.02$).

300   Among the individual PLFAs, no significant differences were found between the N-fixing and

301   non-N-fixing species. Several PLFAs showed a species within tree type effect. Generally, we found

302   that most of the PLFAs were more abundant under *A. dealbata* than *A. implexa* and *E. camaldulensis*,

303   whereas the amount of PLFA under *E. polyanthemos* was intermediate between them (Table 6). This

304   trend was also reflected in the total amount of PLFA (Table 6). The fungal-to-bacterial (F:B) ratio did

305   not differ between N-fixers and non-N-fixers ($P = 0.64$, Table 5), but some difference among the

306   species was found. The F:B ratio was significantly lower under *A. dealbata* compared with *A. implexa*

307   ($P = 0.03$) , but neither differed significantly from the non-N-fixers. Ignoring tree type and species,

308   there was a significant but very weak positive correlation (R^2 = 0.16, P = 0.01) correlation between

309   C:N ratio and F:B ratio of all samples, whereas no correlation was found between F:B ratio and total

310   N (R^2 = -0.02 , P = 0.57), or total C (R^2 = -0.03, P = 0.93)**.** Total fungal PLFA was not correlated with C:N

311   ratio (R^2 = -0.02, P = 0.68) but weakly positively correlated to total N (R^2 = 0.29, P < 0.01) and total C

312   (R^2 = 0.34, P < 0.01).

313

314   **4. Discussion**

315   There were indications that the N fixed by the N-fixing trees was redistributed and utilized

316   by the non-N-fixing trees. Overall, there was a strong species effect within the N-fixing tree types,

317   whereas the non-N-fixing species were more similar to each other. Characterization of the soil

318   microbial community showed no differences among the N-fixers and the non-N-fixers, but some

319   differences in communities under different tree species.

320

321   *4.1. Nitrogen cycling*

322   The total amount of N in the leaves and total litter of N-fixing trees was significantly higher than

323   that of non-N-fixing trees (Table 3). In addition, total N content of the soil was significantly higher

324   under *A. implexa*, compared with the other tree species (Table 4). Similarly, δ^{15}N value was

325   significantly higher in both soil layers under *A. implexa* compared with the other tree species (Table

326   2). Values of δ^{15}N in soil and leaves found here were higher compared with other studies in south

327   eastern Australia (-2 ‰ to 3 ‰, Forrester et al., 2007; May and Attiwill, 2003) but are within the

328   range of values reported globally (-7 ‰ to 15 ‰, Pörtle et al., 2007; Roggy et al., 1999; Shearer and

329   Kohl, 1986). The other studies in Australia measured rotational plantations, whereas our site had

330   been pasture for many decades prior to planting. Use of fertilizer and/or livestock manure in the

331   previous pasture may have increased the initial soils' δ^{15}N values (Watzka et al., 2006).

332   Atmospheric N has a δ^{15}N value of 0 ‰, whereas N pools in the soil have a higher δ^{15}N value

333   (between 5 – 6 ‰ at this site). Therefore, we expected to find a lower (diluted with atmospheric N)

334   δ^{15}N value in leaves and species-specific litter of the N-fixing species (and the soils below them)

335   compared with the non-N-fixing species. However, we found no significant difference in δ^{15}N values

336   between the N-fixing and non-N-fixing trees (Table 1). Several possible mechanisms could explain

337   this finding. As available N content was low in the soil at this site (Hoogmoed et al., 2014), the non-

338 N-fixing tree species may be able to take up organic forms ('unavailable') of N from the litter layer

339 (Averill and Finzi, 2011; Schimel and Bennett, 2004), which has a significantly lower $\delta^{15}N$ value

340 compared with the soil (Table 2). Another explanation could be that the N-fixing tree species are not,

341 or only at low rates, fixing atmospheric N due to low available phosphorus (e.g., Batterman et al.,

342 2013). The N-fixing and non-N-fixing trees would then share the same primary N source (e.g. bulk

343 soil or organic N from the litter layer) and this could explain the similar values of $\delta^{15}N$ in leaves and

344 species-specific litter. However, it could also be that the non-N-fixing trees have access to the N that

345 was fixed from the atmosphere by the N-fixing trees. To explore if the $\delta^{15}N$ signature measured

346 under both tree types was a result of fixed atmospheric N, we collected soil from a large patch of

347 non-N-fixing trees assuming there was negligible influence of N-fixing trees. The $\delta^{15}N$ value of this

348 reference soil, was indeed higher (6.1 ‰) compared with when both tree types were growing close

349 together. While these samples were collected at a later time, the results suggests that the lower

350 value of $\delta^{15}N$ in soil under both tree types is associated with similar access to fixed atmospheric N

351 from the N-fixing trees.

352   Fixed atmospheric N can be redistributed in ecosystems and acquired by non-N-fixers through a

353 number of possible pathways (Figure 2). The most commonly suggested pathway (Figure 2, pathway

354 1-2-3) is via the bulk soil pool that contains atmospheric N released from decomposed N-fixer litter

355 and dead roots (e.g. Forrester et al., 2006; May and Attiwill, 2003; van Kessel et al., 1994). If the non-

356 N-fixing trees at the site took up N primarily from the bulk soil, the $\delta^{15}N$ value of their biomass would

357 be more similar to the $\delta^{15}N$ value of the soil, whereas the $\delta^{15}N$ value of the N-fixers biomass would

358 be more similar to that of the atmosphere (0‰, Shearer and Kohl, 1986). However, as the $\delta^{15}N$ value

359 in leaves of both N-fixers and non-N-fixers were similar and significantly lower than that of the soil,

360 this suggests the non-N-fixing trees were not taking N solely from the bulk soil. We propose the non-

361 N-fixing trees may have been able to access the fixed atmospheric N pool directly after it has been

362 fixed by the N-fixers and before is cycled through the tree biomass and to the soil through

363 decomposing litter (Figure 2, pathway 1-4). The roots of the N-fixers and non-N-fixers may be closely

364   connected, physically or possibly by mycorrhizal fungi (He et al., 2003), so when N-fixers roots slough

365   cells that are mineralized, the non-N-fixer rapid takes up the newly fixed mineral N. With such tight

366   N cycling, these small-scale rhizosphere processes may not reduce the δ^{15}N of the bulk soil. Similarly,

367   in another study, in the first three years after planting, a significantly higher concentration of N was

368   found in fine roots of *Eucalyptus* when grown together with *Acacia* than when grown in

369   monoculture, suggesting that N transfer was occurring belowground before litter was input to the

370   soil (Khanna (1997).

371   ^{15}N natural abundance studies are complicated due to isotopic fractionation processes that

372   occur during the (biochemical) cycling of N in the system (Robinson, 2001), which can mask

373   differences in N sources among the tree species. Our experimental design allowed us to examine the

374   pathways of fixed N but not to quantify the amount of fixed N in a mixed-species planting. Isotopic

375   fractionation has been observed during biochemical N cycling processes in the soil (e.g. Templer et

376   al., 2007), as well as during uptake by plants and allocation to different plant tissues (e.g. Gathumbi

377   et al., 2002). However, it is often hypothesized that in soils with low available N, such as our planting

378   (8.6 kg ha-1 in the 0-20 cm soil layer, Hoogmoed et al., 2014), little to no fractionation during N

379   uptake by plants takes place, as the plants will utilize all available N sources, irrespective of their

380   isotopic composition (Högberg et al., 1996). Furthermore, we assume that possible differences in

381   fractionation during translocation within the tree, among different tree species will be negligible.

382   The mycorrhizal status of a plant can also cause fractionation and affect the δ^{15}N of its biomass

383   (Högberg et al., 1996; Zeller et al., 2007). *Acacia* and *Eucalyptus* species form symbiotic relationships

384   with both arbuscular (Adjoud-Sadadou and Halli-Hargas, 2000; Birhane et al., 2013; Chilvers et al.,

385   1987) and ectomycorrhizal fungi (Chilvers et al., 1987; Diagne et al., 2013; Jumpponen et al., 2004).

386   Many mycorrhizal fungi are not host-specific, and different tree species within a forest often have

387   associations with the same species of mycorrhizal fungi (He et al., 2003). To our knowledge, the

388   exact species of mycorrhizal fungi that may colonize the study tree species are unknown or whether

389   they are host specific. The possibility of different rates of isotopic fractionation due to association

390   with different mycorrhizal fungi species is possible.

391   Resorption of N from leaves during senescence was not significant for N-fixers and non-N-fixers

392   based on comparison of concentration of total N in leaves and species-specific litter (Table 3).

393   However, N concentration was significantly lower in species-specific litter than leaves in *A. implexa*

394   only, indicating significant resorption of N. Values of δ^{15}N were slightly lower in the species-specific

395   litter compared with leaves for both N-fixing and non-N-fixing tree types suggesting some

396   fractionation of δ^{15}N during senescence. In contrast, in 10-year-old mixed-species plantation, a

397   significantly lower δ^{15}N in litter compared with leaves was found in *Acacia mearnsii* but not the co-

398   occurring *Eucalyptus globulus* Forrester et al. (2007). These species-specific responses underline the

399   importance of measuring δ^{15}N in leaves, litter and soil, when studying the cycling of fixed N in forest

400   systems. Here, the δ^{15}N value of the total litter, which contained litter inputs from various

401   surrounding tree species, was not significantly different among the tree types or tree species, so the

402   soil under each tree species received litter with the same δ^{15}N value. The higher δ^{15}N value of the

403   total litter pool compared with the species-specific litter, although only significant for *E.*

404   *polyanthemos*, may be because in addition to the leaves, the total litter pool includes twigs, bark

405   and pods, which can have a higher δ^{15}N value (Ståhl et al., 2005; Templer et al., 2007) and different

406   decomposition rates.

407   While not statistically significant, the δ^{15}N value in the leaves among some of the tree species

408   differed. The δ^{15}N value of *E. camaldulensis* was higher (but not significantly, $P = 0.11$) than that of *A.*

409   *dealbata*. This could indicate that the N pool accessed by *E. camaldulensis* contains less

410   atmospherically-fixed nitrogen compared with *A. dealbata*. Interestingly, soils beneath *A. dealbata*

411   had a significantly higher δ^{15}N value compared with the other tree species, despite no differences in

412   litter δ^{15}N inputs. This could point to lower litter inputs into the soil under *A. dealbata,* which would

413   cause less dilution of the soils δ^{15}N value (i.e. a higher δ^{15}N value compared with the other tree

414   species). However, we found that soil underneath *A. dealbata* contained almost double the amount

415   of total C and total N, compared with the other tree species, which indicates either higher litter

416   and/or root inputs or lower uptake of these nutrients, and would cause a lower $\delta^{15}N$ value under *A.*

417   *dealbata*. One potential explanation is rapid nitrogen cycling under *A. dealbata,* which was found at

418   this site (Hoogmoed et al., 2014) and is common under N-fixing trees (e.g. Boyle et al., 2008; Kaye et

419   al., 2000). Many N-cycling processes discriminate against the heavier ^{15}N isotope and use the lighter

420   ^{14}N. This results in higher levels of ^{15}N in the soil, as the ^{14}N-enriched end products are more prone

421   to leave the soil via plant uptake, leaching or volatilization (Pörtle et al., 2007; Templer et al., 2007).

422   Taken together, our results suggest that there was facilitation by N-fixers by supplying N to non-

423   N-fixers in this relatively young tree planting. Likely pathways by which the non-N-fixing trees

424   acquired this newly fixed N include through root interactions between the tree types or via

425   utilization of organic forms of nitrogen from the litter layer, instead of indirectly from decomposed

426   litter inputs, or decreased competition for soil available N. This means that even in a dry climate

427   where litter decomposition is a slow process (as was the case at our study site, *ca.* 600 mm yr^{-1}), the

428   inclusion of N-fixing trees in a mixed species forest may provide fast, short-term benefits in terms of

429   N supply to non-N-fixing trees.

430

431   *4.2. Microbial communities*

432   Overall, the soil microbial community composition, as measured by PLFAs, was not significantly

433   different under N-fixing and non-N-fixing trees (P = 0.07, Figure 1). To our knowledge, few studies

434   have compared microbial communities under N-fixing and non-N-fixing tree species (Bini et al., 2013;

435   Boyle et al., 2008) but some insights have been gained. Similarly, there was no difference in

436   microbial community composition between the non-N-fixing *Pseudotsuga menziesii* (Douglas fir) and

437   N-fixing *Alnus rubra* (red alder) trees in forest of north-western North America (Boyle et al. (2008) or

438   in microbial biomass C or N under *Acacia mangium* compared with *Eucalyptus grandis* in a 20-

439   month-old mixed-species planting in Brazil (Bini et al., 2013). However, there was significantly more

440   dehydrogenase enzyme activity under *A. mangium* than *E. grandis,* suggesting some differences in

441   the microbial community composition or activity underneath these N-fixing and non-N-fixing tree

442   species Differences in microbial community have been in ecosystems invaded by exotic N-fixers,

443   which may have a larger effect on the microbial community than native species (e.g Allison et al.,

444   2006; Lorenzo et al., 2010; Remigi et al., 2008).

445   Regardless of tree species, any difference in microbial community among the soil samples

446   from our site was most strongly correlated ($R^2 > 0.5$) with total amount of PLFA, followed by total C

447   and total N in the 0-10 cm soil layer (Figure 1). Increasing amounts of total C and N indicate

448   increasing amounts of organic substrate for microbial growth (i.e., total amount of PLFA), which has

449   been found in previous studies to be correlated with microbial biomass C (Bailey et al., 2002a;

450   Potthoff et al., 2006).

451   Although no differences in microbial communities were found under N-fixer and non-N-

452   fixers, some differences were found at the species level. The soil microbial community in soil under *A.*

453   *dealbata* trees was significantly different to that of the other tree species ($P \leq 0.02$, Figure 1).

454   Furthermore, the amount of several specific PLFAs were significantly higher under *A. dealbata* (Table

455   6), contributing to the significantly higher total amount of PLFA underneath *A. dealbata* compared

456   with the other tree species. The higher amounts of PLFA under *A. dealbata* further support the

457   mechanism of higher nutrient cycling rates (i.e. higher microbial activity due to larger microbial

458   population) which may accelerate ^{15}N fractionation processes and explain the high δ^{15}N value of the

459   soil under *A. dealbata*.

460   We hypothesised a decrease in the F:B ratio under N-fixing trees due to increased levels of

461   soil N (Rachid et al., 2013), as N content of the soil is often negatively correlated with F:B ratio in

462   forest ecosystems (Högberg et al., 2007)*.* However, there was no difference in F:B ratio between N-

463   fixing and non-N-fixing trees, or among individual tree species at the site. Regardless of tree type or

464   species, no correlation was found between F:B ratio and total N ($R^2 < 0.01$), total C ($R^2 < 0.01$) or C:N

465   ratio ($R^2 = 0.16$). A meta-analysis found a positive correlation between F:B and C:N, but only when

466   the C:N ratio was higher than 18.4 (Waring et al., 2013). The C:N ratio in our soils was lower and the

467   small range of C:N ratios (11.0 – 15.5) may not have provided sufficient spread in the data to detect

468   a relationship between these factors. Total fungal PLFA was slightly better correlated to total C and

469   total N (P < 0.01, $R^2 = 0. 34$ and 0.29 respectively). While the amount of PLFA is a proxy for microbial

470   biomass, and should only cautiously be considered for microbial activity, our results may correspond

471   to Bailey et al. (2002b), who found that fungal activity rather than F:B ratio was positively correlated

472   with soil C.

473

474 **5. Conclusion**

475 The results presented here suggest that even in a young planting in a dry environment (< 800 mm yr^{-}

476 ^{1}) where litter decomposition is slow, N-fixers may play an important role in facilitation of non-N-

477 fixing trees. Possible pathways by which non-N-fixing trees could take up newly fixed N include

478 direct below-ground exchange of fixed atmospheric N from N-fixing trees to the non-N-fixing trees,

479 or via the uptake of organic forms of N from the litter layer, instead of via the slower process of

480 decomposition of litter from N-fixers. While both N-fixing tree species appeared to fix atmospheric N,

481 they were substantially different in terms of C and N addition to the soil, as well as microbial

482 community composition. *Acacia dealbata* had significantly higher levels of soil C and N and a larger

483 microbial mass compared with the other N fixer *A. implexa*. This shows that the effect of N-fixing

484 tree species on soil carbon sequestration is species-specific, cannot be generalized and requires

485 planting trails to determine if there will be benefits to carbon sequestration.

486

487

500

501

502 **References**

503 Adjoud-Sadadou, D., Halli-Hargas, R., 2000. Occurrence of arbuscular mycorrhizaon aged *Eucalyptus*.

504   Mycorrhiza 9, 287-290.

505 Allison, S.D., Nielsen, C., Hughes, R.F., 2006. Elevated enzyme activities in soils under the invasive

506   nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry 38, 1537-1544.

507 Australian Bureau of Meteorology, 2011. Australian Government, Bureau of Meteorology.

508 Averill, C., Finzi, A., 2011. Increasing plant use of organic nitrogen with elevation is reflected in

509   nitrogen uptake rates and ecosystem δ^{15}N. Ecology 92, 883-891.

510 Bailey, V.L., Peacock, A.D., Smith, J.L., Bolten Jr, H., 2002a. Relationships between soil microbial

511   biomass determined by chloroform fumigation-extraction, substrate induced respiration, and

512   phospolipid fatty acid analysis. Soil Biology & Biochemistry 34, 1385-1389.

513 Bailey, V.L., Smith, J.L., Bolton Jr, H., 2002b. Fungal-to-bacterial ratios in soils investigated for

514   enhanced C sequestration. Soil Biology and Biochemistry 34, 997-1007.

515 Batterman, S.A., Wirzbuger, N., Hedin, L.O., 2013. Nitrogen and phosphorus interact to control

516   tropical symbiotic N_2 fixation: a test in *Inga punctata*. Journal of Ecology 101, 1400-1408.

517 Bauhus, J., Paré, D., Côté, L., 1998. Effects of tree species, stand age and soil type on soil microbial

518   biomass and its activity in a southern boreal forest. Soil Biology & Biochemistry 30, 1077-1089.

519 Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic

520   matter in forest systems. Environmental Reviews 5, 1-25.

521 Bini, D., dos Santos, C.A., Bouillet, J.-P., de Morais Gonçalves, J.L., Cardoso, E.J.B.N., 2013. Eucalyptus

522   grandis and Acacia mangium in monoculture and intercropped plantations: Evolution of soil

523   and litter microbial and chemical attributes during early stages of plant development. Applied

524   Soil Ecology 63, 57-66.

525 Binkley, D., Senock, R., Bird, S., Cole, T.G., 2003. Twenty years of stand development in pure and

526   mixed stands of *Eucalyptus saligna* and nitrogen-fixing *Facaltaria moluccana*. Forest Ecology

527   and Management 182, 93-102.

528   Birhane, E., Sterck, F.J., Bongers, B., Kuypers, T.W., 2013. Arbuscular mycorrhizal impacts on

529   competitive interactions between *Acacia etbaica* and *Boswellia papyrifera* seedlings under

530   drought stress. Journal of Plant Ecology, 1-11.

531   Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial

532   communities: effects of agricultural management, season, and soil type on phospolipid fatty

533   acid profiles. Microbial Ecology 36, 1-12.

534   Bouillet, J.-P., Laclau, J.-P., Gonçalves, J.L.d.M., Voigtlaender, M., Gava, J.L., Leite, F.P., Hakamada, R.,

535   Mareschal, L., Mabiala, A., Tardy, F., Levillain, J., Deleporte, P., Epron, D., Nouvellon, Y., 2013.

536   Eucalyptus and Acacia tree growth over entire rotation in single- and mixed-species

537   plantations across five sites in Brazil and Congo. Forest Ecology and Management 301, 89-101.

538   Boyle, S.A., Yarwood, R.R., Bottomley, P.J., Myrold, D.D., 2008. Bacterial and fungal contributions to

539   soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology &

540   Biochemistry 40, 443-451.

541   Bray, J.R., Curtis, J.T., 1957. An ordination of upland forest communitites of southern Wisconsin.

542   Ecological Monographs 27, 325-349.

543   Burger, B., Reich, P., Cavagnaro, T.R., 2010. Trajectories of change: riparian vegetation and soil

544   conditions following livestock removal and replanting. Australian Ecology 35, 980-987.

545   Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain

546   litter decay responses to simulated nitrogen deposition. Ecology 81, 2359-2365.

547   Chapin, F.S., Walker, L.R., Fastie, C.L., Sharman, L.C., 1994. Mechanisms of primary succession

548   following deglaciation at Glacier Bay, Alaska. Ecological Monographs 64, 149-175.

549   Chazdon, R.L., 2008. Beyond deforestation: restoring forests and ecosystem services on degraded

550   lands. Science 320, 1458-1460.

551   Chilvers, G.A., Lapeyrie, F.F., Horan, D.P., 1987. Ectomucorrhizal vs endomycorrhizal fungi within the

552   same root system. New Phytologist 107, 441-448.

553 Cunningham, S.C., Metzeling, K.J., Mac Nally, R., Thomson, J.R., Cavagnaro, T.R., 2012. Changes in

554   soil carbon of pastures after afforestation with mixed species: sampling, heterogeneity and

555   surrogates. Agriculture Ecosystems & Environment 158, 58-65.

556 Diagne, N., Thioulouse, J., Sanguin, H., Prin, Y., Krasova-Wade, T., Sylla, S., Galiana, A., Baudoin, E.,

557   Neyra, M., Svistoonoff, S., Lebrun, M., Duponnois, R., 2013. Ectomycorrhizal diversity

558   enhances growth and nitrogen fixation of *Acacia mangium* seedlings. Soil Biology &

559   Biochemistry 57, 468-476.

560 Fennessy, M.S., Cronk, J.K., 1997. The effectiveness and restoration potential of riparian ecotones for

561   the management of nonpoint source pollution, particularly nitrate. Critical Reviews in

562   Environmental Science and Technology 27, 285-317.

563 Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through

564   two soil depth profiles. Soil Biology and Biochemistry 35, 167-176.

565 Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in

566   belowground ocmmunities. Ecology Letters 12, 1238-1249.

567 Forrester, D.I., 2014. The spatial and temporal dynamics of species interactions in mixed-species

568   forests: From pattern to process. Forest Ecology and Management 312, 282-292.

569 Forrester, D.I., Bauhus, J., Cowie, A.L., Vanclay, J.K., 2006. Mixed-species plantations of Eucalyptus

570   with nitrogen-fixing trees: a review. Forest Ecology and Management 233, 211-230.

571 Forrester, D.I., Schortemeyer, M., Stock, W.D., Bauhus, J., Khanna, P.K., Cowie, A.L., 2007. Assessing

572   nitrogen fixation in mixed- and single species plantations of *Eucalyptus globulus* and *Acacia*

573   *mearnsii*. Tree Physiology 27, 1319-1328.

574 Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and

575   fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

576 Galiana, A., Gnahoua, G.M., Chaumont, J., Lesueur, D., Prin, Y., Mallet, B., 1998. Improvement of

577   nitrogen fixation in *Acacia mangium* through inoculation with rhizobium. Agroforestry

578   Systems 40, 297-307.

579   Gathumbi, S.M., Cadisch, G., Giller, K.E., 2002. 15N natural abundance as a tool for assessing N2-

580   fixation of herbaceous shrub and tree legumes in improved fallows. Soil Biology &

581   Biochemistry 34, 1059-1071.

582   Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global

583   Change Biology 8, 345-360.

584   Harrison, K.A., Bardgett, R.D., 2010. Influence of plant species and soil conditions on plant-soil

585   feedback in mixed grassland communitites. Journal of Ecology 98, 384-395.

586   Harrison, S.R., Herbohn, J.L., Tisdell, C.A., Lamb, D., 2000. Timber production and biodiversity

587   tradeoffs in plantation forestry, In: Oats, W.E., Folmer, H. (Eds.), Sustainable Small-Scale

588   Forestery: Socio-Econogic Analysis and Policy. Edward Elgar Publishing Ltd, Cambridge, pp. 65-

589   76.

590   He, X.-H., Critchley, C., Bledsoe, C., 2003. Nitrogen transfer within and between plants through

591   common ycorrhizal networks (CMNs). Critical Reviews in Plant Sciences 22, 531-567.

592   Henriksen, T.M., Breland, T.A., 1999. Nitrogen availablitiy effects on carbon mineralization, fungal

593   and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil

594   Biology & Biochemistry 31, 1121-1134.

595   Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7,

596   336-339.

597   Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest

598   soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590-601.

599   Högberg, P., Högbom, L., Schinkel, H., Högberg, M., Johannisson, C., Wallmark, H., 1996. [15]N

600   abundance of surface soils, roots and mucorrhizas in profiles of European forest soils.

601   Oecologia 108, 207-214.

602   Hoogmoed, M., Cunningham, S.C., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2014. Is there more soil

603   carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species

604   restoration plantings? Agriculture, Ecosystems & Environment 188, 80-84.

605   Hoogmoed, M., Cunningham, S.C., Thomson, J.R., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2012.

606   Does afforestation of pastures increase sequestration of soil carbon in Mediterranean climates?

607   Agriculture Ecosystems & Environment 159, 176-183.

608   Hossain, A.K.M.A., Raison, R.J., Khanna, P.K., 1995. Effects of fertilizer application and fire regime on

609   soil microbial biomass carbon and nitrogen, and nitrogen mineralization in an Australian

610   subalpine eucalypt forest. Biology and Fertility of Soils 19, 246-252.

611   Jumpponen, A., Claridge, A.W., Trappe, J.M., Lebel, T., Claridge, D.L., 2004. Ecological relationships

612   among hypogeous fungi and trees: inference from association analysis integrated with habitit

613   modeling. Mycologia 96, 510-525.

614   Kaye, J.P., Resh, S.C., Kaye, M.W., Chimner, R.A., 2000. Nutrient and carbon dynamics in a

615   replacement series of *Eucalyptus* and *Albizia* trees. Ecology 81, 3267-3273.

616   Khanna, P.K., 1997. Comparison of growth and nutrition of young monocultures and mixed stands of

617   *Eucalyptus globulus* and *Acacia mearnsii*. Forest Ecology and Management 94, 105-113.

618   Knoke, T., Ammer, C., Stimm, B., Mosandl, R., 2008. Admixing broadleaved to coniferous tree species:

619   a review on yield, ecological stability and economics. European Journal of Forest Research 127,

620   89-101.

621   Lorenzo, P., Rodríguez-Echeverría, S., Gonzáles, L., Freitas, H., 2010. Effect of invasive *Acacia*

622   *dealbata* Link on soil microorganisms as determined by PCR-DGGE. Applied Soil Ecology 44,

623   245-251.

624   May, B.M., Attiwill, P.M., 2003. Nitrogen-fixation by *Acacia dealbata* and changes in soil properties 5

625   years after mechanical disturbance or slash-burning following timber harvest. Forest Ecology

626   and Management 181, 339-355.

627   Minoshima, H., Jackson, L.E., Cavagnaro, T.R., Sánchez-Moreno, S., Ferris, H., Temple, S.H., Goyal, S.,

628   Mitchell, J.P., 2007. Soil food webs and carbon dynamics in response to conservation tillage in

629   california. Soil Science Society of America Journal 71, 952-963.

630   Mosse, K.P.M., Patti, A.F., Smernik, R.J., Christen, E.W., Cavagnaro, T.R., 2012. Physicochemical and

631   microbiological effects of long- and short-term winery wastewater application to soils. Journal

632   of Hazardous Materials 201-202, 219-228.

633   Munro, N.T., Fischer, J., Wood, J., Lindenmayer, D.B., 2009. Revegetation in agricultural areas: the

634   development of structural complexity and floristic diversity. Ecological Applications 19, 1197-

635   1210.

636   Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L.,

637   Spolymos, P., Stevens, M.H.H., Wagner, H., 2013. The Vegan Package, 2.0-7 ed.

638   Parrotta, J.A., 1999. Productivity, nutrient cycling, and succession in single- and mixed-species

639   plantations of *Casuarina equisetifolia, Eucalyptus robusta*, and *Leucaena leucocephala* in

640   Puerto Rico. Forest Ecology and Management 124, 45-77.

641   Paul, K.I., Polglase, P.J., Nyakuengama, J.G., Khanna, P.K., 2002. Change in soil carbon following

642   afforestation. Forest Ecology and Management 168, 241-257.

643   Pörtle, K., Zechmeister-Boltenstert, S., Wanek, W., Ambus, P., Berger, T.W., 2007. Natural ^{15}N

644   abundance of soil N pools and N_2O reflect the nitrogen dynamics of forest soils. Plant and Soil

645   259, 79-94.

646   Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M., Joergensen, R.G., 2006. Soil

647   microbial community composition as affected by restoration practices in California grassland.

648   Soil Biology & Biochemistry 38, 1851-1860.

649   Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester

650   more carbon in forest soils? Biogeochemistry 101, 133-149.

651   Priha, O., Grayston, S.J., Hiukka, R., Pennanen, T., Smolander, A., 2001. Microbial community

652   structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Pica abies*

653   and *Betula pendula* at two forest sites. Biology and Fertility of Soils 33, 17-24.

654   R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for

655   Statistical Computing, Cienna, Austria.

656   Rachid, C.T.C.C., Balieiro, F.C., Peixoto, R.S., Pinheiro, Y.A.S., Piccolo, M.C., Chaer, G.M., Rosado, A.S.,

657   2013. Mixed plantations can promote microbial integration and soil nitrate increases with

658   changes in the N cycling genes. Soil Biology & Biochemistry 66, 146-153.

659   Remigi, P., Faye, A., Kane, A., Deruaz, M., Thioulouse, J., Cissoko, M., Prin, Y., Galiana, A., Dreyfus, B.,

660   Duponnois, R., 2008. The exotic legume tree species *Acacia holosericea* alters microbial soil

661   functionalities and the structure of the arbuscular mycorrhizal community. Applied and

662   Environmental Microbiology 74, 1485-1493.

663   Resh, S.C., Binkley, D., Parrotta, J.A., 2002. Greater soil carbon sequestration under nitrogen-fixing

664   trees compared with *Eucalyptus* species. Ecosystems 5, 217-231.

665   Robinson, D., 2001. δ15N as an integrator of the nitrogen cycle. Trends in Ecology and Evolution 16,

666   153-162.

667   Roggy, J.C., Prévost, M.F., Gourbiere, F., Casabianca, H., Garbaye, J., Domenach, A.M., 1999. Leaf

668   natural ^{15}N abundance and total N concentration as potential indicators of plant N nutrition in

669   legumes and pioneer species in a rain forest of French Guiana. Oecologia 120, 171-182.

670   Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology

671   85, 591-602.

672   Schweiter, J.A., Madritch, M.D., Felkert-Quinn, E., Bailey, J.K., 2012. From genes to ecosystems: plant

673   genetics as a link between above- and belowground processses, In: Wall, D.H., Bardgett, R.D.,

674   Behan-Pelletier, V., Herrick, J.E., Jones, T.H., Ritz, K., Six, J., Strong, D.R., van der Putten, W.H.

675   (Eds.), Soil Ecology and Ecosystem Services. Oxford University Press, Oxford.

676   Shearer, G., Kohl, D.H., 1986. N_2-Fixation in field settings: estimations based on natural [15]N

677   abundance. Australian Journal of Plant Physiology 13, 699-756.

678   Siddique, I., Engel, V.L., Parrotta, J.A., Lamb, D., Nardoto, G.B., Ometto, J.P.H.B., Martinelli, L.A.,

679   Schmidt, S., 2008. Dominance of legume trees alters nutrient relations in mixed species forest

680   restoration plantings within seven years. Biogeochemistry 88, 89-101.

681 Ståhl, L., Högberg, P., Sellstedt, A., Buresh, R.J., 2005. Measuring nitrogen fixation by *Sesbania*

682 *sesban* planted fallows using ^{15}N tracer technique in Kenya. Agroforestry Systems 65, 67-79.

683 Team, R.C., 2012. R: A language and environment for statitcal computing, R Foundation for Statistical

684 computing, 3.0.0 ed, Vienna, Austria.

685 Templer, P.H., Arthur, M.A., Lovett, G.M., Weathers, K.C., 2007. Plant and soil natural abundance

686 δ^{15}N: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. Oecologia

687 153, 399-406.

688 van der Heiden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes

689 as driveres of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11,

690 296-310.

691 van Kessel, C., Farrell, R.E., Roskoski, J.P., Keane, K.M., 1994. Recycling of the naturally-occuring ^{15}N

692 in an established stand of *Leucaena leucocephala*. Soil Biology and Biochemistry 26, 757-762.

693 Vitousek, P.M., Walker, L.R., 1989. Biological invasion by Myrica faya in Hawaii: plant demography,

694 nitrogen fixation, ecosystem effects. Ecological Monographs 59, 247-265.

695 Wang, F., Li, Z., Xia, H., Zou, B., Li, N., Liu, J., Zhu, W., 2010. Effects of nitrogen-fixing and non-

696 nitrogen-fixing tree species on soil properties and nitrogen transformation during forest

697 restoration in southern China. Soil Science and Plant Nutrition 56, 297-306.

698 Wardle, D.A., 2002. Communities and ecosystems: linking the aboveground and belowground

699 components. Princeton University Press, Princeton.

700 Waring, B.G., Averill, C., Hawkes, C.V., 2013. Differences in fungal and bacterial physiology alter soil

701 carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecology

702 Letters 16, 887-894.

703 Watzka, M., Buchgraber, K., Wanek, W., 2006. Natural ^{15}N abundance of plants and soils under

704 different management practices in a montane grassland. Soil Biology & Biochemistry 38, 1564-

705 1576.

706   Zeller, B., Brechet, C., Maurice, J.-P., Le Tacon, F., 2007. ^{13}C and ^{15}N isotopic fractionation in trees,

707   soil and fungi in a natural forest stand an a Noway spruce plantation. Annals of Forest Science

708   64, 419-429.

709

710

Figures

Figure 1. Non-metric multidimensional scaling (NMDS) ordination of range standardized PLFAs from

surface (0-10 cm) soil samples under different tree species. ■ : *A. dealbata*, ⬟: *A. implexa*, ◇ : *E.*

camaldulensis, ▽ : *E. polyanthemos* . Stress value was 0.07. Figure contains only significant vectors

that explain more than 50% of the variation. Vector length represents the relative magnitude of

explained variation and the direction indicates that of a positive increase.

Figure 2. Possible pathways of fixed nitrogen (N) cycling in a mixed-species forest.

1) On the left, atmospheric N is fixed by N-fixing bacteria in the root nodules of N-fixing trees (e.g.

Acacia spp). 2) The fixed N is used by the N-fixing trees for biomass production and is transported to

various tree tissues. 3) After leafs are shed and decomposed the fixed N is included in the soil N pool

underneath the canopy of the N-fixing trees. 4) Non-N-fixing trees (e.g. *Eucalyptus* spp) can take up

this fixed N from the soil N pool. 5) Alternatively, the non-N-fixing trees may extend their roots to

closely intertwine with the N-fixing trees' roots. Any fixed N that is released by the N-fixers' roots via

root exudates or after root death and subsequent N mineralization, is taken up directly by the non-

N-fixing trees. 6) After leaf abscission of the non-N-fixing trees and subsequent decomposition of

litter and dead roots, the soil pool underneath the non-N-fixing trees will contain atmospherically

fixed N, 7) which the non-N-fixing trees can again take up.

Tables

731

732 Table 1. Nested- and one-way-ANOVA results, comparing $\delta^{15}N$ (‰) value. Nested ANOVAs were

733 performed on tree types and tree species nested within tree type, separate for each sample type:

734 leaves, species-specific litter, litter, soil 0-10cm and soil 10-20 cm. One-way-ANOVA were performed

735 among sample types, separate for each tree species: *Acacia dealbata*, *A. implexa*, *Eucalyptus*

736 *camaldulensis* and *E. polyanthemos*. A significant ($P < 0.05$) difference is indicated by an asterisk (*).

737

738

739 Table 2. Means ± standard errors of $\delta^{15}N$ (‰) in the leaves, species-specific litter, litter, 0-10 cm and

740 10-20 cm soil layer of the individual tree species. Different letters indicate a significant difference (P

741 < 0.05) among tree species (N = 10, compare letters horizontally).

742

743

744 Table 3. Nested- and one-way-ANOVA results, comparing total N (%), total C (%) and C:N ratio.

745 Nested-ANOVA is performed on tree types and tree species nested within tree type, separate for

746 each sample type: leaves, species-specific litter, litter, soil 0-10cm and soil 10-20 cm. One-way-

747 ANOVA was performed among sample types, separate for each tree species: *Acacia dealbata, A.*

748 *implexa, Eucalyptus camaldulensis* and *E. polyanthemos*. A significant ($P < 0.05$) difference is

749 indicated by an asterisk (*). N.b. One way-analysis for sample type comparing Total N and Total C

750 does not include contents in the 0-10 and 10-20 cm soil layers, as these could not be compared due

751 to different measurement units.

752

753   Table 4. Means and standard errors of total N and total C and C:N ratio in the leaves, species-specific

754   litter, litter, 0-10 cm and 10-20 cm soil layer of the individual tree species. Different letters indicate

755   a significant difference ($P < 0.05$) among tree species ($N = 10$, compare letters horizontally).

756

757

758   Table 5. Nested-ANOVA results, comparing individual PLFAs, total PLFA and Fungal-bacterial ratio,

759   between tree types and species nested within tree type. A significant ($P < 0.05$) difference is

760   indicated by an asterisk (*).

761

762

763   Table 6. Means ($\mu g\ g^{-1}$ dry soil) and standard error of PLFAs and fungal-to-bacterial ratio in the 0 - 10

764   cm soil layer under the individual tree species. Different letters indicate a significant ($P < 0.05$)

765   difference among the tree species (N = 10, compare letters horizontally).

766

Table 1.

	test	F	P
Sample type			
Leaves	Tree type	7.50	0.11
	Tree type (species)	0.32	0.73
Species-specific litter	Tree type	0.26	0.66
	Tree type (species)	0.92	0.41
Litter	Tree type	0.29	0.65
	Tree type (species)	1.77	0.18
Soil 0-10 cm	Tree type	0.14	0.75
	Tree type (species)	7.38	< 0.01*
Soil 10-20 cm	Tree type	0.01	0.93
	Tree type (species)	12.5	< 0.01*
Species			
A. dealbata	Sample type	53.0	< 0.01 *
A. implexa	Sample type	9.64	< 0.01 *
E. camaldulensis	Sample type	36.2	< 0.01 *
E. polyanthemos	Sample type	45.8	< 0.01 *

Table 2.

Sample type	*Acacia dealbata*	*Acacia implexa*	*Eucalyptus camaldulensis*	*Eucalyptus polyanthemos*
Leaves	1.87 ± 0.23 [a]	2.06 ± 0.66 [a]	2.85 ± 0.41 [a]	2.41 ± 0.28 [a]
Species-specific litter	1.54 ± 0.28 [a]	1.31 ± 0.66 [a]	2.00 ± 0.21 [a]	1.25 ± 0.34 [a]
Litter	2.28 ± 0.37 [a]	1.86 ± 0.46 [a]	1.88 ± 0.26 [a]	2.75 ± 0.31 [a]
Soil 0-10 cm	5.55 ± 0.29 [a]	4.39 ± 0.22 [b]	4.83 ± 0.18 [b]	4.67 ± 0.16 [b]
Soil 10-20 cm	5.80 ± 0.27 [a]	4.17 ± 0.28 [c]	4.98 ± 0.22 [b]	5.15 ± 0.13 [ab]

Table 3.

	Test	Total N		Total C		C:N ratio	
		F	P	F	P	F	P
Sample type							
Leaves (%)	Tree type	33.7	0.03*	1.63	0.33	17.0	0.05
	Tree type (species)	4.36	0.02*	0.25	0.78	6.00	<0.01*
Species-specific	Tree type	11.0	0.08	< 0.01	0.97	5.18	0.15
litter (%)	Tree type (species)	4.90	0.01*	3.40	0.04*	16.9	< 0.01*
Litter (%)	Tree type	26.8	0.04*	0.14	0.74	41.7	0.02*
	Tree type (species)	0.62	0.54	0.11	0.90	0.32	0.73
Soil 0-10 cm	Tree type	1.05	0.41	0.97	0.43	0.69	0.49
(t ha^{-1}) [c]	Tree type (species)	17.3	< 0.01*	14.0	< 0.01*	6.73	< 0.01*
Soil 10-20 cm	Tree type	1.23	0.38	0.18	0.71	4.77	0.16
(t ha^{-1}) [c]	Tree type (species)	5.17	0.01*	4.64	0.02*	3.89	0.03*
Tree type							
A. dealbata	Sample type	18.2	< 0.01 *	5.18	0.01 *	37.5	< 0.01 *
A. implexa	Sample type	48.5	< 0.01 *	4.76	0.02 *	64.2	< 0.01 *
E. camaldulensis	Sample type	7.28	< 0.01 *	6.01	< 0.01 *	37.2	< 0.01 *
E. polyanthemos	Sample type	17.1	< 0.01 *	1.74	0.20	67.8	< 0.01 *

Table 4.

Sample type	*Acacia dealbata*	*Acacia implexa*	*Eucalyptus camaldulensis*	*Eucalyptus polyanthemos*
Total N				
Leaves (%)	2.84 ± 0.14 [a]	2.68 ± 0.11 [a]	1.64 ± 0.12 [b]	1.21 ± 0.07 [c]
Species-specific litter (%)	2.13 ± 0.23 [a]	1.85 ± 0.09 [a]	1.26 ± 0.10 [b]	0.70 ± 0.05 [c]
Litter (%)	1.44 ± 0.09 [a]	1.33 ± 0.09 [a]	1.05 ± 0.11 [b]	0.97 ± 0.06 [b]
Soil 0-10 cm (t ha^{-1})	6.47 ± 0.65 [a]	3.36 ± 0.19 [b]	3.01 ± 0.20 [b]	3.58 ± 0.30 [b]
Soil 10-20 cm (t ha^{-1})	2.84 ± 0.21 [a]	1.98 ± 0.25 [b]	1.76 ± 0.16 [b]	2.06 ± 0.18 [b]
Total C				
Leaves (%)	49.2 ± 0.88 [a]	49.8 ± 1.04 [a]	49.3 ±1.73 [a]	48.0 ± 1.69 [a]
Species-specific litter (%)	50.3 ± 2.14 [a]	46.0 ± 1.31 [a]	50.1 ± 1.14 [a]	46.5 ± 1.30 [a]
Litter (%)	43.9 ± 1.21 [a]	44.6 ± 1.35 [a]	44.3 ± 0.69 [a]	44.5 ± 0.95 [a]
Soil 0-10 cm (t ha^{-1})	75.8 ± 6.96 [a]	43.9 ± 2.76 [b]	38.3 ± 2.89 [b]	48.4 ± 3.97 [b]
Soil 10-20 cm (t ha^{-1})	32.2 ± 2.52 [a]	23.1 ± 3.09 [b]	22.3 ± 2.23 [b]	28.4 ± 2.17 [ab]
C:N ratio				
Leaves	17.7 ± 0.87 [a]	18.8 ± 0.47 [a]	32.0 ± 3.02 [b]	40.7 ± 1.67 [c]
Species-specific litter	25.4 ± 1.84 [a]	25.09 ± 0.86 [a]	42.5 ± 3.41 [b]	69.5 ± 5.24 [c]
Litter	32.1 ± 2.80 [a]	35.4 ± 2.64 [a]	45.6 ± 4.32 [b]	47.9 ± 4.10 [b]
Soil 0-10 cm	11.8 ± 0.17 [a]	13.1 ± 0.36 [bc]	12.6 ± 0.25 [ab]	13.6 ± 0.42 [c]
Soil 10-20 cm	11.3 ± 0.25 [a]	11.7 ± 0.22 [a]	12.5 ± 0.36 [a]	14.2 ± 0.71 [b]

Table 5.

	test	F	Pr
14:0	Tree type	0.73	0.48
	Tree type (species)	12.1	< 0.01 *
i15:0	Tree type	0.09	0.79
	Tree type (species)	3.49	0.04 *
a15:0	Tree type	0.80	0.47
	Tree type (species)	12.9	< 0.01 *
3-OH 14:0	Tree type	4.59	0.17
	Tree type (species)	1.68	0.20
i16:0	Tree type	0.69	0.49
	Tree type (species)	4.37	0.02 *
16:1ω7cis	Tree type	0.65	0.51
	Tree type (species)	6.57	< 0.01 *
16:0	Tree type	0.59	0.52
	Tree type (species)	11.3	< 0.01 *
i17:0	Tree type	0.35	0.61
	Tree type (species)	3.35	< 0.04 *
17:0cy	Tree type	0.57	0.53
	Tree type (species)	5.62	< 0.01 *
17:0	Tree type	0.94	0.44
	Tree type (species)	1.46	0.25
2-OH 16:0	Tree type	0.06	0.83
	Tree type (species)	4.60	0.02 *
18:2ω6,9 all cis	Tree type	0.27	0.66
	Tree type (species)	0.80	0.46
18:1ω9cis	Tree type	0.70	0.49
	Tree type (species)	5.31	< 0.01 *
18:1ω9trans	Tree type	0.65	0.50
	Tree type (species)	8.71	< 0.01 *
19:0cy	Tree type	0.91	0.44
	Tree type (species)	1.25	0.30
20:0	Tree type	0.29	0.64
	Tree type (species)	4.38	0.02 *
Total PLFA	Tree type	0.51	0.55
	Tree type (species)	6.60	< 0.01 *
Fungal:bacterial ratio	Tree type	0.29	0.64
	Tree type (species)	2.67	0.08

Table 6.

	Acacia dealbata	*Acacia implexa*	*Eucalyptus camaldulensis*	*Eucalyptus polyanthemos*
14:0	1.02 ± 0.09 [a]	0.53 ± 0.07 [b]	0.47 ± 0.04 [b]	0.63 ± 0.09 [b]
i15:0	4.30 ± 0.63 [a]	2.87 ± 0.35 [b]	3.02 ± 0.27 [b]	3.67 ± 0.33 [ab]
a15:0	3.29 ± 0.33 [a]	1.67 ± 0.21 [b]	1.55 ± 0.11 [b]	1.92 ± 0.21 [b]
3-OH 14:0	0.56 ± 0.11 [a]	0.37 ± 0.10 [ab]	0.12 ± 0.06 [b]	0.27 ± 0.10 [b]
i16:0	3.00 ± 0.30 [a]	2.00 ± 0.30 [b]	1.96 ± 0.17 [b]	2.20 ± 0.18 [b]
16:1ω7cis	3.96 ± 0.42 [a]	2.31 ± 0.24 [b]	2.10 ± 1.35 [b]	2.74 ± 0.41 [b]
16:0	10.6 ± 0.94 [a]	6.16 ± 0.64 [b]	6.03 ± 0.41 [b]	7.19 ± 0.63 [b]
i17:0	1.01 ± 0.16 [a]	0.67 ± 0.08 [b]	0.67 ± 0.05 [b]	0.79 ± 0.08 [ab]
17:0cy	2.45 ± 0.26 [a]	1.60 ± 0.20 [b]	1.50 ± 0.10 [b]	1.86 ± 0.19 [b]
17:0	0.65 ± 0.14 [a]	0.53 ± 0.08 [a]	0.37 ± 0.07 [a]	0.58 ± 0.10 [a]
2-OH 16:0	0.49 ± 0.09 [a]	0.22 ± 0.06 [b]	0.25 ± 0.06 [b]	0.38 ± 0.07 [ab]
18:2ω6,9 all cis	3.49 ± 0.91 [a]	2.77 ± 0.41 [a]	2.58 ± 0.26 [a]	3.20 ± 0.26 [a]
18:1ω9cis	5.96 ± 0.78 [a]	3.70 ± 0.46 [b]	3.66 ± 0.26 [b]	4.06 ± 0.33 [b]
18:1ω9trans	5.99 ± 0.59 [a]	3.54 ± 0.36 [b]	3.15 ± 0.27 [b]	4.22 ± 0.51 [b]
19:0cy	2.15 ± 0.46 [a]	1.84 ± 0.26 [a]	2.02 ± 0.25 [a]	2.63 ± 0.20 [a]
20:0	0.34 ± 0.03 [a]	0.26 ± 0.03 [bc]	0.23 ± 0.02 [c]	0.31 ± 0.03 [ab]
Total PLFA	49.2 ±5.48 [a]	31.0 ± 3.52 [b]	29.7 ± 2.12 [b]	36.7 ± 3.28 [b]
Fungal:bacterial ratio	0.17 ± 0.04 [a]	0.26 ± 0.02 [b]	0.24 ± 0.02 [ab]	0.24 ± 0.01 [ab]