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Agriculture Ecosystems & Environment, 2014; 188:80-84

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Final publication at http://dx.doi.org/10.1016/j.agee.2014.02.013

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18 August 2021

http://hdl.handle.net/2440/84608

Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings?

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Abstract

Afforestation of agricultural land provides an important opportunity to mitigate climate change by storing carbon (C) in both plant biomass and the soil. Here we present results of a study in which we sought to determine whether soil under nitrogen(N)-fixing trees contained more C than soil under non-N-fixing trees in mixed-species plantings, and thus if inclusion of N-fixers is beneficial in terms of increasing soil C sequestration. Soils were sampled directly beneath N-fixing and non-N-fixing tree species in riparian and upland mixed-species plantings in southeastern Australia. Soil C and N contents were assessed at both the landscape and individual planting scales. At the landscape scale, there were higher levels of soil C and N under N-fixing trees compared with non-N-fixing trees. At the individual planting scale, the patterns were less clear with both large increases and decreases occurring across the range of sites. The results presented here indicate that the inclusion of N-fixers may help to increase soil C, and N, but that the response may be site– and species–specific.

Keywords: *Acacia*, afforestation, carbon sequestration, *Eucalyptus*, nitrogen cycling, riparian

1. Introduction

Forests store an estimated 80% of all above-ground terrestrial carbon (C) and 40% of all below-ground terrestrial C (Dixon *et al.*, 1994). Afforestation of agricultural land provides an important opportunity for mitigating climate change (IPCC, 2007). Management strategies such as afforestation increase the vast, relatively stable, long–term sink of C within the soil. Soil C sequestration after afforestation can take several decades to increase soil C substantially (e.g., Hoogmoed *et al.*, 2012). Consequently, it is important to determine how management of tree plantings can be altered to accelerate soil C sequestration.

Increasing the soil C sequestration potential of afforestation requires an understanding of the processes involved in sequestration and how management alters these process. For example, the tissues of the tree species planted may differ in their chemical composition (e.g., Osono and Takeda, 2005) and, consequently, in their effect on organic matter decomposition and subsequent C sequestration. Effects of different tree species on soil C sequestration have not been widely assessed (e.g., Vesterdal *et al.*, 2008), but there is increasing evidence that soil C sequestration is higher under N-fixing trees (e.g., Resh *et al.*, 2002). N-fixing trees are often included in tree plantations to improve above-ground productivity through facilitation (Siddique *et al.*, 2008). However, most observations are from single-species plantations, and there is little understanding of the role of N-fixers in mixed-species plantings (e.g., Kasel *et al.*, 2011).

In addition to the species planted, climate, soil type and management practices affect the soil C sequestration potential of afforestations (Paul *et al.*, 2002). Within a region, landscape position may also alter soil C sequestration. Afforestation of riparian zones may lead to large amounts of sequestered C (Burger *et al.*, 2010), as well as an increase in the recalcitrance of the soil C pool (Smith *et al.*, 2012), because riparian zones are generally wetter and more fertile than upland areas. However, such soil conditions may promote faster rates of biogeochemical cycling, and hence C loss via heterotrophic respiration, compared with drier, less fertile upland areas (Smith *et al.*, 2012).

Here, we present results of a study focusing on ecological restoration plantings, which include several native tree species, are not harvested, and therefore, the soil is largely undisturbed after planting. The extent of restoration plantings is increasing due to their potential additional environmental benefits, including providing habitat for native flora and fauna (Munro *et al.*, 2009) and, in riparian zones, buffering streams against the impacts of agriculture (Brooks and Lake, 2007). We study two fundamental issues when establishing mixed-species restoration plantings to maximize soil carbon sequestration: 1) Which tree species should be planted? and 2) Where in the landscape should the trees be planted? Our specific questions were:

1) Is there more total N, available N and, consequently, more total C in soil under N-fixing trees compared with non-N-fixing trees?

2) Is there a difference in soil response (in terms of C and N stocks) to N-fixers between riparian and upland positions?

We hypothesised that both C and N content in the soil would be higher under N-fixing trees, and that the difference between N-fixers and non-N-fixers would be less at the riparian sites due to their higher fertility, reducing the relative benefits of N-fixation.

2. Materials and methods

2.1 Study area

A field survey was conducted in four young tree plantings near Benalla (36.5538 °S, 145.9815 °E) in northern Victoria, Australia, in November 2011. Prior to European settlement, the region was covered in open woodlands (10-30 m tall, 10-30% projective foliage cover Specht, 1981) dominated by *Eucalyptus* species with grassy understoreys. Since European settlement in the 1840's the region has been extensively cleared for agriculture, including cereal crops and pasture for livestock. The climate across the region is temperate with seasonal changes in mean monthly maximum temperature (12.6–31.8 °C) and minimum temperature (3.1–15.2 °C), and a winter-dominant annual precipitation (670-715 mm year-1, Bureau of Meteorology, 2013)

2.2 Site selection and sampling

This study was conducted at mixed-species restoration plantings. Sites were selected that had never before been harvested, were regenerated from tube stock on former agricultural pastures, >15 yr of age, had a minimum area of 1 ha and had to contain an adequate number of N-fixing and non-N-fixing trees. This resulted in four study sites. Understory vegetation was sparse and low (<0.5 m) or absent. At each site, 20 N-fixing trees and 20 non-N-fixing trees were selected randomly. This number was chosen to make sure the whole planting was sampled as evenly as possible. The tree species sampled and other key site attributes are provided in the appendix, Table A1. Two of the four plantings were located in riparian zones (sites R1 and R2), with streams ~2 m wide and the other two plantings were located in upland sites (sites U1 and U2).

Soil was sampled using an auger from the 0-10 cm and 10-20 cm soil layers under the crown (within 0.25 - 1 m of the trunk) of the selected trees, as these soil layers generally contain most soil organic C in forest systems (Jobbágy and Jackson, 2000). Litter or grassy vegetation was removed from the surface before sampling. No pronounced organic layer was present at any of the sites. In the 0-10 cm layer, four subsamples (~100 g) were taken and then bulked to make one composite sample (per tree), in order to compensate for spatial heterogeneity. In the 10-20 cm layer, two subsamples (~200 g) were taken to make one composite sample (per tree). Fewer subsamples were taken in this layer as the soil was hard and difficult to sample. The soil was stored immediately at 4^{o}C for 2 days until further processing upon return to the laboratory (see below). Soil bulk density samples were taken with a 96 cm^3 core at both depth layers, under six of the N-fixing trees and six of the non-N-fixing trees at each site, making sure that sampled points were spread across the whole site. The diameter of the stem of each tree was measured at breast height (approx. 1.3 m).

2.3 Sample processing

Soil samples were passed through a 2 mm sieve. A sub-sample of the fresh soil was immediately used for determination of NO_3^{-}N and NH_4^+-N concentrations, and rates of potential mineralizable N (PMN) by anaerobic incubation, as follows. Inorganic N was extracted from 7 g of fresh soil with 2M KCL and then measured colorimeterically using a modified method reported in Miranda *et al.* (2001) for NO_3^- (plus NO_2^-) and Forster (1995) for NH_4^+. For PMN determination, 10 mL deionized water was added to 7 g of fresh soil in a 50 mL centrifuge tube, and the head space of the tube was filled with N_2 (Waring and Bremner, 1964). The soil was incubated for 7 days at 37°C. Then, 10 mL of 4M KCL was added to the water and NH_4^+ was analyzed as described above. The rate of PMN of the soil was expressed as the difference between the NH_4^+ extracted from fresh soil and the NH_4^+ extracted after the 7-day incubation. Another soil sub-sample was air–dried, ground to a fine powder using a mill (IKA, Malaysia) and then total C and total N measured by dry combustion in an ANCA GSL 2 elemental analyzer (Sercon Ltd., UK).

Bulk density samples were dried at 105°C for 48 h. Any stones in the bulk density samples were retained to determine their volume using displacement of water in a measuring cylinder. Bulk density was calculated by dividing the oven-dried soil mass by the steel cylinder volume less the stone volume. Total C, total N, NH_4^+, NO_3^- and PMN concentrations were converted to soil contents (t ha^{-1}) for data analysis and presentation.

2.4 Statistical analysis

The soil survey was statistically analysed as a split-plot design with the four plantings treated as separate plots, site position in the landscape (i.e., riparian or upland) as the effect among sites, and tree type (N-fixer or non-N-fixer) as the effect within sites (*N* = 160 trees). When a significant interaction effect was found (landscape x tree type), the data were re–analysed as separate one-way ANOVAs for riparian and upland sites, using tree type as the fixed factor, and site as a random factor (*N* = 80 trees). Comparison of tree types within individual sites was tested using one-way ANOVAs (*N* = 40 trees). All data analyses were performed in SYSTAT version 10.

3. Results and Discussion

We found that soil C and N were influenced by sample depth, tree type and landscape position in complex ways (Table 1). At the landscape scale, in the 10-20 cm soil layer there was a significantly higher level of total C under the N-fixing trees compared with the non-N-fixing trees, and marginally higher levels ($P = 0.06$) of total N (Table 1), although overall levels of soil C and N in this soil layer are relatively low (see Fig. 1c and d). There was no difference in total C and N between the tree types in the 0-10 cm soil layer (Table 1). However, the C:N ratio in the 0-10 cm soil layer was significantly lower under N-fixers compared with non-N-fixers ($P < 0.01$). Higher contents of soil N were expected under N-fixing trees, as N-fixing trees often have higher N content in their biomass, litter and root exudates. Decomposition of their litter is likely to elevate N content in the soil (Forrester *et al.*, 2007). The higher C content under the N-fixing trees may be due to increased N fertility resulting in faster growth of trees and consequently increased (below-ground) C inputs to the soil (e.g., Resh *et al.*, 2002). In addition, high levels of soil N slow down the decomposition of the recalcitrant fraction (lignin) of organic matter (Berg and Matzner, 1997).

Except for PMN, the different forms of mineral N (NH_4^+, NO_3^-, and their sum: total mineral N) showed highly significant differences between N-fixing and non-N-fixing trees, particularly in the 10-20 cm soil layer (Table 1). There was a significant difference between tree types and a significant interaction between tree type and landscape position, indicating higher levels of mineral N under N-fixers compared with non-N-fixers, but only in the riparian landscape position ($P < 0.01$). The same pattern was also found in the 0-10 cm soil layer, but only for NO_3^- ($P < 0.01$). Contrasting findings among sites are consistent with previous studies, which found both increased N mineralization under N-fixers compared with non-N-fixers (e.g., Rhoades *et al.*, 1998; Siddique *et al.*, 2008), and no difference between N-fixers and non-N-fixers (e.g., Wang *et al.*, 2010). However, none of these studies were conducted in riparian zones. The soil C:N ratio has been found to be negatively correlated with N mineralization rates (Booth *et al.*, 2005). At the riparian sites where the overall C:N ratio is lower than the upland sites (online supplementary material Table S2), the difference in N mineralization between the tree types was more pronounced and may explain the different response at the riparian and upland sites. The higher levels of mineral N under N-fixers in the riparian sites suggest more accessible N for plant growth. In this way, N-fixers can facilitate the growth of neighbouring non-N-fixing trees (Siddique *et al.*, 2008). Temporal measurements of available N stocks were highly variable while rates of N mineralization (PMN) were more stable in a riparian zone of our study region (Smith *et al.*, 2012) which could explain a lack of relationship between soil C and available N stocks in these sites.

Contents of total N were expected to be higher in the riparian sites (irrespective of tree species) compared with the upland sites, because of their higher soil fertility and moisture (e.g., Smith *et al.*, 2012) but this was not the case at our study sites (Table 1). Our hypothesis predicting less benefit from N-fixers at riparian sites compared with the upland sites, due to predicted higher levels of N at the riparian sites, could therefore not be tested in this study.

Further analysis at the individual site level revealed important variability among the sites (Fig.1, appendix Table A2) and helped explain some of the patterns found in the overall statistical model described above. For example, significant differences in total C and N were found between tree types in the 0-10 cm soil layer at some sites. Specifically, more total C and N were found under N-fixers at two of the sites (R1, $P < 0.01$ and U1, $P \leq 0.01$), whereas less total C ($P < 0.01$) and marginally less total N ($P = 0.06$) were found under N-fixers at site U2. At site R2, no differences were found in total C and N between tree types (Fig. 1A and B). In the 10-20 cm soil layer, where the landscape-scale analysis showed significantly more C and N under N-fixers, the difference at the individual site level was not significant at any of the sites. However, a (non-significant) trend for higher total C and N under the N-fixers can be observed (Fig. 1 C and D). Several studies of single-species plantations have found higher C and N contents under N-fixers compared with non-N-fixers (e.g., Resh *et al.*, 2002; Ussiri *et al.*, 2006; Wang *et al.*, 2010). Our results indicate that the effect of N-fixers on soil C and N in young mixed-species restoration plantings is variable among sites, showing increases, no change, or decreases.

There are several potential hypotheses that might explain why N-fixers did not have higher amounts of soil C and N than non-N-fixers at some sites (R2 and U2). For example, the N-fixing trees at this site may only be fixing negligible amounts of atmospheric N or none at all. A lack of N fixation by N-fixing trees can be caused by low available phosphorus levels (Pearson and Vitousek, 2002) or the absence of specific *Rhizobia* at these particular planting sites. Without the advantage of binding atmospheric N, the N-fixing trees may have lower growth rates and lower C inputs into the soil compared with the non-N-fixing trees. Indeed, at both site R2 and U2, there was no difference in soil N under the N-fixers and the non-N-fixers (Fig. 1). Alternatively, it is possible that the non-N-fixing trees had access to the N fixed by the N-fixing trees. This would increase the growth rate of the non-N-fixers, and hence, litter inputs into the soil and C sequestration. Consequently, differences in C sequestration between N-fixers and non-N-fixers found in single-species plantings plantings may be obscured in mixed-species plantings.

Most studies have tested the effect of N-fixers on C sequestration using a single tree species (Rhoades *et al.*, 1998; Ussiri *et al.*, 2006; Wang *et al.*, 2010). However, tree species, even within the same type (e.g. N-fixing species), may affect soil C due to their different functional traits (e.g., litter quality and quantity, root structure, exudates). This may play an important role in explaining the large difference among the sites.

In conclusion, the ability of N-fixers to increase soil C and N was site specific suggesting it is important to determine which site and species characteristics affect the potential of N-fixers to increase soil C. Experimental designs that include different N-fixing and non-N-fixing species, or meta-analyses of the available data of different tree species should be utilised to make stronger generalizations about the effects of N-fixing trees on soil C sequestration. Furthermore, in the current study, soil was sampled directly underneath the crowns of individual trees. More research is required to determine if the (potential) increases in soil C under N-fixing trees have a significant effect on soil C sequestration at the whole planting and landscape scales.

Acknowledgements

This research was funded by the Australian Research Council Linkage Program (LP0990038), Goulburn Broken Catchment Management Authority (CMA), North Central CMA, Victorian Department of Sustainability and Environment, EPA Victoria and Kilter Pty. Ltd. T.R.C. (FT120100463), J.B. (FT110100602) and P.J.B. (FT120100715) were supported by Australian Research Council Future Fellowships. M.H. thanks the Holsworth Wildlife Research Endowment for additional funding for fieldwork and laboratory analysis. We thank Mr Scott McDonald for his assistance and good cheer in the field. Thanks also to the landholders for access to their properties and Dr. Willem Hoogmoed for a critical reading of the manuscript. Many thanks to the two anonymous reviewers and editor of AgEE for their thoughtful comments that helped improved this paper.

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Tables

Table 1. Results of split-plot ANOVA for potential mineralizable nitrogen (PMN), ammonium (NH_4^+), nitrate (NO_3^-), total mineral N, total N, total C and the carbon to nitrogen ratio (C:N), in the 0-10 cm and 10-20 cm soil layer. *N* = 160 trees.

Figures

Fig. 1. Total C in the 0-10 cm (a) and 10-20 cm (c) soil layer and total N in the 0-10 cm (b) and 10-20 cm soil layer (d) for each individual planting site (R: Riparian, U: Upland), under N-fixers (black bars) and non-N-fixers (white bars). Capital A and B above the bars indicate a significant difference ($P < 0.05$), small letters indicate a marginal difference ($P < 0.1$), ns: non-significant. Values are means (N = 20 trees) with standard errors indicated by bars.

Table 1.

Variable	**Hypothesis test**	**0-10 cm**		**10-20 cm**	
		F	***P***	***F***	***P***
PMN	Tree type	0.90	0.44	0.05	0.85
	Landscape	16.7	0.06	6.33	0.13
	Tree type x Landscape	0.35	0.61	0.02	0.89
NH_4^+	Tree type	1.97	0.30	346	< 0.01*
	Landscape	0.01	0.92	13.2	0.07
	Tree type x Landscape	0.01	0.90	228	< 0.01*
NO_3^-	Tree type	242	< 0.01*	29.8	0.03*
	Landscape	10.9	0.08	19.4	0.05
	Tree type x Landscape	54.8	0.02*	31.4	0.03*
Total mineral N	Tree type	72.5	0.01*	126	0.01*
	Landscape	5.59	0.14	42.1	0.02*
	Tree type x Landscape	9.03	0.10	102	0.01*
Total N	Tree type	0.83	0.46	16.6	0.06
	Landscape	2.28	0.27	6.40	0.13
	Tree type x Landscape	0.55	0.54	7.63	0.11
Total C	Tree type	0.18	0.71	70.6	0.01*
	Landscape	4.99	0.16	0.04	0.86
	Tree type x Landscape	0.50	0.55	0.41	0.56
C:N	Tree type	294	< 0.01*	0.61	0.52
	Landscape	20.2	0.05	8.27	0.10
	Tree type x Landscape	12.8	0.07	1.44	0.35

* represents a significant (*P* < 0.05) difference

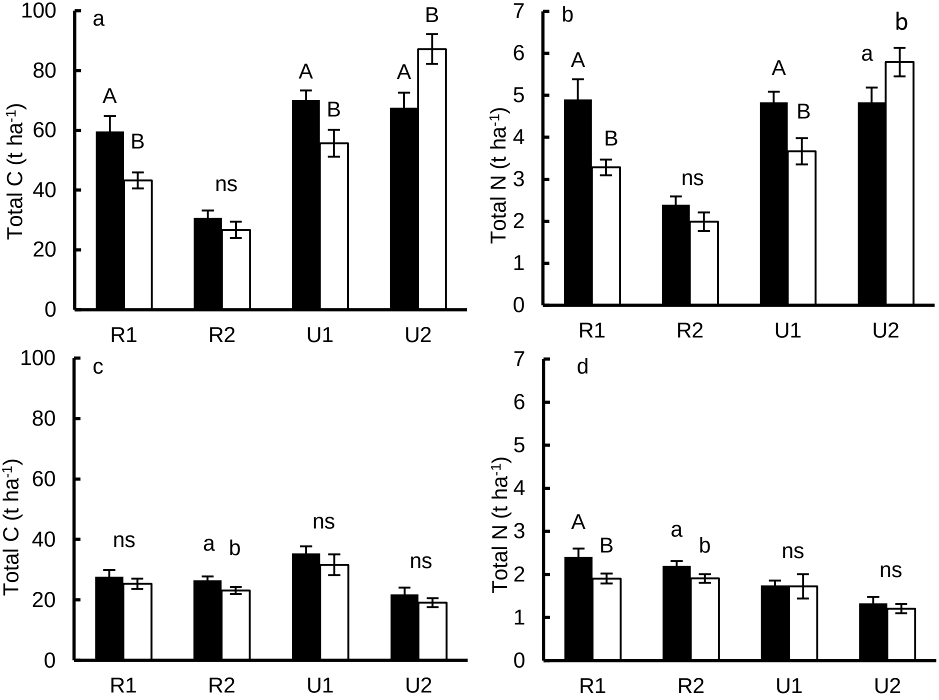
a
b
c
d
Total C (t ha^{-1})
Total N (t ha^{-1})
0
20
40
60
80
100
0
1
2
3
4
5
6
7
R1
R2
U1
U2
A
B
ns
a
b

Table S1. Characteristics of the tree plantings. Values for diameter at breast height (DBH, *N* = 20 trees) are means with standard errors.

	Landscape position	**Age (yr)**	**Location**	**Soil type and texture** [a]	**Sampled tree species**	**DBH** (cm)	**tree density (ha^{-1})**
U1	Upland	18	36.5796 °S,	Sodosol	N-fixers:		*Total: 493*
			146.1046 °E		*Acacia verniciflua* A. Cunn.	12.2 ± 2.8	6.0
				Sandy loam	*Acacia pycnantha* Benth.	17.7 ± 0.8	10.5
					Allocasuarina luehmannii (R. T. Baker) L. A. S. Johnson	13.5 ± 1.4	6.3
					Non-N-fixers:		
					Eucalyptus polyanthemos Schauer	17.3 ± 1.3	61.5
					Eucalyptus sideroxylon A Cunn. Ex Woolls	24.6 ± 2.4	221.0
U2	Upland	15	36.4967 °S,	Sodosol	N-fixers:		*Total: 359*
			146.1435 °E		*Acacia implexa* Benth	20.2 ± 1.6	16.6
				Sandy loam	*Allocasuarina luehmannii* (R. T. Baker) L. A. S. Johnson	16.5 ± 1.0	10.8
					Non-N-fixers:		
					Eucalyptus polyanthemos Schauer	27.8 ± 8.8	58.2
					Eucalyptus sideroxylon A Cunn. Ex Woolls	31.2 ± 2.4	114.8
R1	Riparian	15	36.8621 °S	Chromosol	N-fixers:		*Total: 411*
			145.5800 °E		*Acacia implexa* Benth	13.2 ± 1.5	44.9
				Loam	*Acacia dealbata* Link.	14.0 ± 1.5	77.0
					Non-N-fixers :		
					Eucalyptus camaldulensis Dehnh.	17.8 ± 2.0	140.4
					Eucalypus polyanthemos Schauer	25.5 ± 1.6	57.1
R2	Riparian	18	36.6976 °S	Sodosol	N-fixers:		*Total: 344*
			145.8773 °E		*Acacia melanoxylon* R. Br.	9.5 ± 0.7	129.4
				Sandy loam			
					Non-N-fixers:		
					Eucalyptus camaldulensis Dehnh.	7. 2 ± 0.6	214.6

[a] McKenzie *et al.* (2000)

Table S2. Means ± standard error of soil variables for individual riparian (R) and upland (U) plantings, separated by soil depth and tree type (NF: non-N-fixer, F: N-fixer). *N* = 20 (for bulk density *N* = 6). PMN: potential mineralizable nitrogen.

Site	**Soil depth**	**Tree type**	**Bulk Density**	**NH_4^+ (kg ha^{-1})**	**NO_3^- (kg ha^{-1})**	**PMN (kg ha^{-1})**	**Total mineral N (kg ha^{-1})**	**C:N**
R1	0-10 cm	F	0.97 ± 0.11	3.0 ± 1.0	10.1 ± 1.7*	81.6 ± 13.3*	13.1 ± 2.1*	12.4 ± 0.3
		NF	0.86 ± 0.08	1.7 ± 1.2	5.0 ± 1.5*	31.8 ± 5.5*	6.8 ± 2.3*	13.1 ± 0.6
	10-20 cm	F	1.19 ± 0.08	6.1 ± 1.7*	5.8 ± 1.5*	13.9 ± 2.8	11.9 ± 3.0*	11.5 ± 0.2*
		NF	1.05 ± 0.04	1.1 ± 0.3*	1.4 ± 0.8*	7.9 ± 1.7	2.5 ± 0.8*	13.3 ± 0.4*
R2	0-10 cm	F	0.91 ± 0.04*	0.9 ± 0.7	11.1 ± 1.1*	40.6 ± 5.3	12.1 ± 1.4*	12.9 ± 0.2*
		NF	1.11 ± 0.05*	0.5 ± 0.4	6.3 ± 1.1*	46.9 ± 4.7	6.7 ± 1.2*	13.6 ± 0.2*
	10-20 cm	F	1.10 ± 0.04	6.2 ± 1.0*	7.0 ± 1.0*	16.9 ± 3.0	13.1 ± 1.3*	12.1± 0.2
		NF	1.16 ± 0.02	1.1 ± 0.4*	3.9 ± 0.6*	20.9 ± 2.7	5.0 ± 0.8*	12.2 ± 0.1
U1	0-10 cm	F	1.08 ± 0.08	3.5 ± 1.1	4.9 ± 1.1	26.9 ± 2.6*	8.4 ± 2.0	14.7 ± 0.3
		NF	0.94 ± 0.07	1.2 ± 0.3	3.6 ± 1.0	18.0 ± 1.6*	4.8 ± 1.1	15.6 ± 0.6
	10-20 cm	F	1.18 ± 0.08	0.6 ± 0.2	0.4 ± 0.2	4.4 ± 0.7	1.0 ± 0.5	20.6 ± 1.1
		NF	0.97 ± 0.14	0.4 ± 0.2	0.7 ± 0.4	5.8 ± 1.2	1.1 ± 0.5	20.8 ± 1.3
U2	0-10 cm	F	1.83 ± 0.12	0.9 ± 0.3	2.2 ± 0.6*	14.1 ± 4.1	3.0 ± 0.7	13.9 ± 0.2*
		NF	1.89 ± 0.12	1.1 ± 1.0	0.0 ± 0.0*	12.9 ± 1.7	1.1 ± 1.0	15.0 ± 0.2*
	10-20 cm	F	1.10 ± 0.17	2.3 ± 1.0	0.2 ± 0.2	5.4 ± 1.4	2.4 ± 1.0	16.6 ± 0.6
		NF	1.08 ± 0.08	1.4 ± 0.3	0.0 ± 0.0	3.7 ± 0.9	1.4 ± 0.3	16.1 ± 0.4

* represents a significant difference between N fixers and non-N-fixers per depth layer.