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N-fixing trees in restoration plantings: Effects on nitrogen supply and soil microbial communities

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A B S T R A C T

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,

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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 Kessel et al., 1994), through root exudates, or via interconnected mycorrhizal networks between the trees (He et al., 2003). This facilitative effect of N-fixers on non-N-fixers is important for net primary production, as well as community development (Siddique et al., 2008) and successional processes (Chapin et al., 1994; Vitousek and Walker, 1989). Consequently, the inclusion of N-fixers in mixed species woody plants may have an important impact upon N dynamics in these systems.

The stand-scale consequences of N_2-fixation on soil C sequestration are ultimately driven by the effects of N on soil processes. This may include impacts on soil microbial communities, which play a key role in organic matter decomposition (Wardle, 2002). This process is governed by complex interactions among factors such as litter quantity and quality (nutrient content and chemical structure), soil microbial community composition and several biotic and abiotic factors (e.g. Prescott, 2010). Soil microbial communities are often found to differ among tree species (Priha et al., 2001), presumably, due to differences in litter quality and quantity (Bauhus et al., 1998; Hobbie, 1992; Schweiter et al., 2012). Higher amounts of N in litter and soil under N-fixing trees are likely to have a major effect on the soil microbial community beneath these trees (Allison et al., 2006). For example, higher available nitrogen or a lower C:N ratio under N-fixers may favour bacterial over fungal decomposers (Fierer et al., 2009; Harrison and Bardgett, 2010). Bacteria are generally less adapted to decompose recalcitrant litter as fungi (Henriksen and Breland, 1999; van der Heiden et al., 2008). Therefore, increased N levels under N-fixing trees may shift the microbial community towards bacterial dominance, slowing the rate of decomposition of organic matter and increasing the rate of soil C sequestration. In contrast, fungal biomass is more recalcitrant and fungi have a higher C assimilation efficiency compared with bacteria, therefore a shift towards more bacteria could also result in a reduction of soil C sequestration (Bailey et al., 2002b).

If the potential for N-fixers to increase soil C sequestration in mixed-species afforestation plantings is to be maximized, we need to better understand the role of N-fixers in these plantings. An extensive literature exists on interactions between N-fixing and non-N-fixing trees (e.g., Bouillet et al., 2013; Forrester, 2014), albeit predominantly in relation to tree growth and wood production (e.g. Binkley et al., 2003; Parrotta, 1999) but also soil C sequestration (e.g. Kaye et al., 2000) or nutrient cycling (e.g. Khanna, 1997). However, there is a lack of consensus about how N-fixers and non-N-fixers interact and what drives differences among studies. Further, little is known about the impact of N-fixers on soil microbial communities in mixed-species plantings. Here, we present the results of a field-based study in which we investigated two important aspects of restoration plantings including both N-fixing and non-N-fixing tree species: 1) the pathways that fixed atmospheric N takes within the stand and 2) the effect of N-fixers on the soil microbial community. We asked two specific questions:

1. Do non-N-fixing trees have access to N derived from the fixation of atmospheric N_2 by neighbouring N-fixing trees, in the early development of a tree planting?
2. Do changes in the N dynamics associated with N-fixing trees, result in changes in soil microbial communities in a mixed-species restoration planting?

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

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., *Acacia implexa* Benth, *Acacia melanoxylon* R. Br., and the non-N-fixers were *Eucalyptus camaldulensis* Dehnh., *Eucalyptus polyanthemos* Schauer, *Eucalyptus macrorhyncha* F. Muell, *Eucalyptus 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.

2.2. Sampling

The two dominant N-fixing tree species, *A. dealbata* and *A. implexa*, and the two dominant non-fixing tree species *E. 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 bulked to make one composite sample. In the 10–20 cm layer, two samples (*ca* 200 g) were collected to make one composite sample. Given limited differences δ^{15}N among the tree types (see results), we collected additional soil samples from a large patch of non-N-fixing trees to provide a reference value for δ^{15}N in soil with negligible influence of N-fixing trees. In June 2013, five soil samples were collected in the patch from the 0–10 cm layer, which was *ca* 10 m away from the nearest N-fixing tree. All soil, from both sampling campaigns was stored immediately at 4 °C for 2 days until further processing in the laboratory. Soil bulk density samples were taken at both depth layers, under six of the N-fixers and six of the non-fixers, making sure that trees were spread across the whole sampling area, following Minoshima et al. (2007).

To assess the presence of fixed atmospheric N in litter and fresh leaves, standing litter was collected from within a randomly placed 20 cm × 20 cm quadrat underneath the crown of each tree, within