Introduction of Dalbergia Odorifera Enhances Nitrogen Absorption on Eucalyputs Through Stimulating Microbially Mediated Soil Nitrogen-cycling

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Research Article

Keywords: Eucalyptus plantations, Soil physical-chemical properties, Microbial biomass, Soil enzyme activities, Nitrogen availability

Posted Date: July 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-683628/v1

Abstract

Background: There is the substantial evidence that *Eucalyptus* for nitrogen (N) absorption and increasing the growth benefit from the introduction of N-fixing species, but the underlying mechanisms for microbially mediated soil N cycling remains unclear.

Methods: We investigated the changes of soil pH, soil water content (SWC), soil organic carbon (SOC), total N (TN), inorganic N (NH_4^+-N and NO_3^--N), microbial biomass and three N-degrading enzyme activities as well as the biomass and N accumulation of *Eucalyptus* between a pure *Eucalyptus urophylla* × *grandis* plantation (PP) and a mixed *Dalbergia odorifera* and *Eucalyptus* plantation (MP) in Guangxi Zhuang Autonomous Region, China.

Results: Compared with the PP site, soil pH, SWC, SOC and TN in both seasons were significantly higher at the MP site, which in turn enhanced microbial biomass and the activities of soil N-degrading enzymes. The stimulated microbial activity at the MP site likely accelerated soil N mineralization, providing more available N (NH_4^+-N in both seasons and NO_3^--N in the wet-hot season) for *Eucalyptus* absorption. Overall, the N accumulation of *Eucalyptus* at the MP site was increased by 19.7% and 21.9%, promoting the biomass increases of 15.1% and 19.2% in the dry-cold season and wet-hot season, respectively.

Conclusion: Our results reveal the importance of microbially mediated soil N cycling in the N absorption on *Eucalyputs*. Introduction of *D. odorifera* can enhance N absorption and growth on *Eucalyputs*, improve soil N availability and increased soil C sequestration, which hence can be considered to be an effective sustainable management option of *Eucalyptus* plantations.

Background

Eucalyptus is one of the most extensively planted commercial plantation timber genera in the world (Lino et al. 2016). Currently, most *Eucalyptus* plantations are grown as monocultures and have been intensively managed in short-rotation with continuous cropping (Huang et al. 2017). However, due to high nitrogen (N) consumption with successive rotations, the monoculture plantations of *Eucalyptus* have rapidly depleted soil nutrients (especially N) and water (Liu et al. 1998; Sicardi et al. 2004). The massive application of N fertilizer is common to ensure high and sustainable stand production (Laclau et al. 2005), but this not only increases economic costs but also leads to soil acidification, compaction, contamination of groundwater, and other negative impacts on the environment (Goncalves et al. 1997). Consequently, seeking an optimal silvicultural practice to achieve N sustainable management of *Eucalyptus* plantations is in urgent need.

The N-fixing trees have been widely considered as being important in balancing N losses due to timber harvesting and in reducing the demand for fertilizer application in *Eucalyptus* plantations (May and Attiwill 2003). The growth of N-fixing plants relies largely on the fixed atmospheric-N that accounts for 10% − 90% of the N used by the N-fixing species (Nygren and Leblanc 2015). Therefore, introduction of N-fixing species into *Eucalyptus* plantations would decrease the N uptake of trees from soil, in turn increasing soil N retention (Bouillet et al. 2008; Koutika et al. 2019). In addition, the N can be transferred from N_2-fixing species to *Eucalyptus*, which allows *Eucalyptus* to benefit directly from symbiotic N fixation (Paula et al. 2015; Yao et al. 2019, 2021). In recent years, introducing N-fixing species to improve N absorption and productivity on *Eucalyptus* has been a popular focus of research, but the experimental results remain contradictory depending on the selected N-fixing species and stand conditions (Forrester et al. 2006; Firn et al. 2007; Bouillet et al. 2013). Moreover, because of lack of understanding the underlying mechanisms of soil N cycling, it is still hard to predict how combinations of the N-fixing species and specific sites can lead to the best benefits, making the extension of this silvicultural practice remains difficult around the world.

The advantages of *Eucalyptus* mixed with N_2-fixing tree species are usually attributed to the increase in soil organic N source (Paula et al. 2015; Yao et al. 2019). However, it is important to note that most of these increased organic N sources due to the introduction of N-fixing species becoming available to *Eucalyptus* have to be transformed to inorganic N by the microbial decomposition of the plant tissues and soil organic matter (Versini et al. 2016). Shift of microbial activity under the mixed plantation of *Eucalyptus* and N-fixing species would alter soil N cycling and availability, which ultimately affect the N absorption on *Eucalyputs* (Huang et al. 2014). Microorganisms involve soil biogeochemical cycling mainly through producing specific extracellular enzymes, which has been confirmed by extensive researches (Huang et al. 2017). Soil N-degrading enzymes including leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG) and Urease can serve as indicators of energy N demand (Schimel et al. 2017), which catalyze terminal reactions to depolymerize organic N (Sinsabaugh et al., 2008). Therefore, detecting the change of microbial biomass and soil N-degrading enzyme activities will help us to better understand the underlying mechanisms for microbially mediated soil N cycling under the mixed plantations of *Eucalyptus* and N-fixing species.

Eucalyptus has been introduced and cultivated in south China since the 1970s, and the area of *Eucalyptus* plantations has reached 4.6 million ha by 2014, of which almost half in Guangxi Zhuang Autonomous Region, China (Zhao et al. 2018). In recent years, a variety of N-fixing species such as *Acacia mangium* and *D. odorifera* were introduced to improve N absorption and productivity on *Eucalyptus* planations in Guangxi (Huang et al. 2014; Yao et al. 2019). Here we conducted a mixed plantation of *Eucalyptus* (*E. urophylla* × *E. grandis*) and *D. odorifera* (MP) and a *Eucalyptus* pure plantation (PP) in a long-term field experiment. Previous work in pot experiment has observed a percentage of 6.6–13.6% N transfer from *D. odorifera* to *Eucalyptus* and an increase of 20.4–33.2% in the dry matter yields of *Eucalyptus* compared to the monoculture plantation (Yao et al. 2019). In this study, we investigated the changes of soil pH, soil water content (SWC), soil organic carbon (SOC), total N (TN), inorganic N (NH_4^+-N and NO_3^--N), microbial biomass and three N-degrading enzyme activities as well as the biomass and N accumulation of *Eucalyptus* between PP and MP sites. We would focus on the role of microbial function in driving soil N availability in the dry-cold and wet-hot seasons. Specifically, we hypothesized that the introduction of *D. odorifera* would increase soil organic matter (SOC and TN) and improve soil environment (pH and SWC), which in turn stimulated microbial activities (biomass and N-degrading enzyme activities) and improved N availability (NH_4^+-N and NO_3^--N), ultimately enhancing *Eucalyptus* N absorption and productivity.

Materials And Methods

Experimental design

The study site was located at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry (22°07' N, 106°93' E), Pingxiang City, Guangxi Zhuang Autonomous Region, China. The mean annual precipitation is around 1400 mm, falling mainly from April through September (Wang et al., 2010) and mean annual temperature is 21°C. The soils were formed from granite, classified as red soil in Chinese soil classification, equivalent to oxisol in USDA Soil Taxonomy.

In March 2015, a complete randomized block design with three replicates was established to compare a pure *E. urophylla* × *grandis* plantation (PP) and a mixed species plantation of *D. odorifera* and *E. urophylla* × *grandis* (MP), the proportion in the mixed plantation stands was 1:1 (50E:50D). Three sampling plots (15 m × 15 m) were randomly established within each of the stand type, a 200 m buffer separates the PP and MP plantations. The two species were planted alternately at 2 m spacing in the row, with 2.5 m between rows, giving a total stocking density of 2000 trees ha^{-1}. The fertilizers applied on planting were 140 g N $plant^{-1}$ (urea: $CO(^{14}NH_2)_2$, buried at 20 cm from the each plant), as well as 18 g $plant^{-1}$ K (KCl), 56 g P $plant^{-1}$ (CaH_2PO_4).

Sample of plants collected and N analysis

Five plants in each plot were harvested on March 20 and August 25, 2019, which including below-ground (i.e., roots) and above-ground. The trees of above-ground were separated into components: leaves, branches (living branches and dead branches), stem wood and stem bark. The stem of each tree was sawn into 2 m sections according to Monsic's stratified clip method (You et al. 2018). However, much of the root growth was shallow and lateral, it was difficult to distinguish the fine roots of one tree from those of another. To address this problem, a large soil pit of 1.5 m diameter and 1.0 m depth was excavated around each target tree. All the materials were collected and weighed up fresh weight immediately, and then 500 g of every composition sample was taken in laboratory.

The harvested material was dried at 65°C until constant dry weight period for the biomass analyses. The dried plant material was ground in a ball mill (< 0.1 mm) for the N concentration analyses with a continuous-flow chemical analyzer (AA3) after the degradation using 10 ml H_2SO_4. The biomass and N accumulation of *E. urophylla* × *grandis* in the pot was the sum of leaves, stem, branches, bark and roots.

Sample of soils collected and analysis

On March 19 and August 24, representing the dry-cool season and the wet-hot seasons of the year 2019, respectively, five soil samples were randomly collected from each plot at 0–20 cm depth. During sampling within a plot, the corer was wiped clean of obvious soil particles with paper towel. All samples were stored at -20°Cand sieved < 2 mm to remove visible stones, soil animals, roots and plant materials prior to analysis pH, ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N). Some of the air dried and ball milled sample (sieved < 0.2 mm) were used for the concentration of TN, soil microbial biomass carbon (MBC) and nitrogen (MBN) and SOC analyses.

The pH of the soil samples was measured in a 1:2.5 soil/water suspension. SOC was determined by dichromate oxidation and titration with ferrous ammonium sulfate, the TN concentration was determined by a continuous flow chemical analyzer (AA3) and followed by detection of NH_4^+-N and NO_3^--N. Data was also collected on C/N ratio of soil (C/Nsoil).

MBC and MBN were measured by fumigation-extraction, using 0.5 M K_2SO_4 as the extraction agent (Vance et al. 1987), with a total organic carbon analyzer (1020A; OI, College Station, TX, USA), and were calculated by (Vance et al. 1987).

$MBC = E_C/k_{EC}$ (1)

Where E_C= (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils), and k_{EC}= 0.38.

$MBN = E_N/k_{EN}$ (2)

Where E_N= (organic N extracted from fumigated soils) - (organic N extracted from non-fumigated soils), and k_{EN}= 0.45.

In addition, soil microbial function was expressed by the soil extracellular enzyme activities, involved NAG, LAP and Urease, respectively. We determined the soil enzyme activities using the conventional p-nitrophenol (pNP) assays (Baldrian, 2009) and we took the following specific operation steps: (1) weight 1.25 g fresh soil and add 12.5 mL of 50 mM Sodium acetate buffer, then extract it with using low-energy sonication for 2 min as the soil sample to be tested; (2) take 200 ul soil sample into place microplates and added the 50 ml stock solution to analysis the activity of LAP (stock solution: 200 μM of the L-Leucine-7-amido-4-Methylcoumarin hydrochloride), NAG (stock solution: 200 μM of the 4-MethylumbelliferylN-7-acetyl-β-D-glucosaminide) and Urease (20 mM of the urea), respectively; (3) and then place microplates were incubated for 4 hours in the darkness at 25℃; (4) enzyme assays were performed with a fully automatic full-wavelength enzyme labeling apparatus for an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm. Enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from the sample value. The enzyme activities were measured by 3 replicates for each soil sample and expressed as μmol per gram dry soil and incubation time.

Statistical analyses

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Analysis of Variance (ANOVA) was used to determine the statistical significance (α = 0.05) of tand type, season and their interactive effect on N accumulation and biomass of *Eucalyptus* as well as soil physico-chemical properties and microbial activity. Tukey's multiple comparison test (HSD) was conducted if significant effects of stand type or season was found. Pairwise relationships between plant N accumulation, soil physico-chemical properties and microbial activities were tested using Pearson correlation coefficients.

A structural equation modelling (SEM) approach was also used to test a conceptual model for microbially mediated soil N cycling in the dry-cold and the wet-hot seasons. The SEM analysis was performed with the IBM SPSS Amos 20.0 using the maximum likelihood estimation method. Several tests were used to assess model fit: the Chi-square (c2)-test, comparative fit index (CFI) and root square mean error of approximation (RMSEM).

Results

The biomass and the N content of E. urophylla **×** grandis

The biomass of *Eucalyptus* was significantly by stand type, with greater at the MP than the PP sites in both seasons ($P < 0.05$). At both PP and MP sites, the biomass of *Eucalyptus* in stems was greater than that of the other organs in both seasons (Fig. 1a and b). The N accumulation of the whole plant was significantly affected by season, stand type and their interaction (Table S1). Similar to biomass, the N accumulation of *Eucalyptus* was also significantly greater at the MP than the PP sites in the both seasons (Fig. 1c and d). However, the distribution of N accumulation in different organs was different from that of biomass. Generally, the N accumulation of branch was greater than that of the other organs except for the stems in the both seasons.

Soil physico-chemical properties

Soil pH had no significant difference between the dry-cold and wet-hot season, but was significantly higher at the MP site than at the PP site (Table 1). The concentrations of SOC, TN, NO_3^--N and NH_4^+-N as well as the ratio of SOC to TN (C/N) and SWC were significantly affected by stand type and season. SOC, TN, NH_4^+-N and SWC were significantly higher at the MP than the PP sites and in the wet-hot season than dry-cold season ($p < 0.05$ for all, Table 1). The soil NO_3^--N concentration at the MP site was higher than that at the PP site in the wet-hot season only (Table 1). Compared with the PP site, soil C/N ratio was significantly decreased at the MP site in both seasons (Table 1).

Table 1
Soil Physical chemical properties as affected by introduction N_2-fixing trees and seasons in *Eucalyptus* plantations. PP = pure *Eucalyptus* plantation; MP = mixed with N_2-fixing species and *Eucalyptus* plantation.

Source of variation		pH	SWC	SOC	TN	NO_3^--N	NH_4^+-N	C/N
Dry-cold	MP	4.83 ± 0.12 a	18.63 ± 1.43 b	21.49 ± 3.25 b	1.34 ± 0.13 b	4.22 ± 0.46 b	16.99 ± 3.17 b	16.09 ± 2.03 b
	PP	4.56 ± 0.27 b	16.83 ± 0.86 c	18.96 ± 2.81 c	1.04 ± 0.15 c	4.09 ± 0.36 b	13.55 ± 2.01c	18.78 ± 3.14 a
Wet-hot	MP	4.91 ± 0.16 a	21.48 ± 1.63 a	24.70 ± 3.62 a	1.56 ± 0.15 a	5.94 ± 0.88 a	17.46 ± 1.48 a	16.29 ± 3.69 b
	PP	4.60 ± 0.14 b	19.99 ± 1.11 b	20.85 ± 2.57 b	1.12 ± 0.10 c	4.25 ± 0.27 b	13.59 ± 1.56 c	18.15 ± 0.94 a
Season (S)		ns	64.832***	25.48***	100.852***	87.659***	144.709***	ns
Stand type (T)		7.844*	19.332***	18.631***	9.330**	48.121***	24.905***	7.844*
S × T		ns	ns	ns	8.522**	50.178***	13.667***	ns

Microbial biomass and enzymes activities

Two-way ANOVAs showed that MBC and MBN were significantly affected by stand type and season ($p < 0.05$, Fig. 2), but no significant interactive effect was found ($p > 0.05$, Fig. 2). The MBC and MBN were significantly higher at the MP than the PP sites and in the wet-hot season than dry-cold season (Fig. 2a and b). However, the ratio of MBC/MBN did not significantly differ between the PP and MP sites in the both seasons (Fig. 2c). All of the soil N-degrading enzyme activities were also significantly affected by stand type and season ($p < 0.05$, Fig. 3). Specifically, only the activities of NAG and Urea in the wet-hot season were significantly higher ($p < 0.05$) at the MP than the PP sites (Fig. 3).

Relationships of soil microbial biomass and enzymes activities with soil and plant N pools

Soil pH did not correlated with any other properties in the dry-cold season, while in the wet-hot season it was positively correlated with SWC, SOC, TN, NAG activity and NO_3^--N (Table 2). SWC was positively correlated with MBC, the activities of NAG and Urea, and the N accumulation of *Eucalyptus* in the dry-cold season, and with SOC, TN, NAG activity, NH_4^+-N, NO_3^--N and the N accumulation of *Eucalyptus* in the wet-hot season, respectively (Table 2). SOC also did not correlated with any other properties in the dry-cold season, but in the wet-hot season it was positively correlated with TN, MBC, all of N-degrading enzyme activities, NH_4^+-N, NO_3^--N and the N accumulation of *Eucalyptus* in the wet-hot season. Soil TN content was positively correlated with MBN and the N accumulation of *Eucalyptus* in the dry-cool season, and with MBC, the activities of NAG and Urea, NO_3^--N and the N accumulation of *Eucalyptus* in the wet-hot season, respectively (Table 2). MBC and MBN were positively correlated with all of the N-degrading enzyme activities in the wet-hot season expect for the relationship between MBN and LAP activity (Table 2). MBC was also positively correlated with NH_4^+-N, NO_3^--N and the N accumulation of *Eucalyptus* in the wet-hot season (Table 2). NAG activity was positively correlated with NH_4^+-N, NO_3^--N and the N accumulation of *Eucalyptus* in both seasons (Table 2). LAP activity was positively correlated with NH_4^+-N and the N accumulation of *Eucalyptus* in the wet-hot season but not in the dry-cold season, while Urea activity was positively correlated with NH_4^+-N and the N accumulation of *Eucalyptus* in the dry-cold season but not in the wet-hot season (Table 2). Both NH_4^+-N and NO_3^--N were positively correlated with the N accumulation of *Eucalyptus* in the wet-hot season, while in the dry-cold season only the NH_4^+-N was positively correlated with the N accumulation of *Eucalyptus* (Table 2).

Table 2
The collection among the soil physical-chemical properties, soil enzymes, soil microbial biomass and N content in the plants

Source of variation	pH	SWC	SOC	TN	MBC	MBN	LAP	NAG	Urease	NH_4^+-N	NO_3^--N	PN
pH		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SWC	0.976**		ns	ns	0.813*	ns	ns	0.895*	0.899*	ns	ns	0.946**
SOC	0.824*	0.857*		ns	ns	ns	ns	ns	ns	ns	ns	ns
TN	0.836*	0.87*	0.835*		ns	0.864*	ns	ns	ns	ns	ns	0.818*
MBC	ns	ns	0.888*	0.968**		ns	ns	ns	ns	ns	ns	ns
MBN	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns
LAP	ns	ns	0.866*	ns	0.812*	ns		ns	ns	ns	ns	ns
NAG	0.889*	0.947**	0.931**	0.908*	0.978**	0.825*	0.908**		0.994**	0.96**	0.858*	0.962**
Urease	ns	ns	0.850*	0.855*	0.866*	0.859*	ns	0.861**		0.935**	ns	0.967**
NH_4^+-N	ns	0.859*	0.883*	ns	0.894*	ns	0.94**	0.946**	ns		0.897*	0.877*
NO_3^--N	0.902*	0.910*	0.947**	0.951**	0.952**	ns	ns	0.928*	ns	0.831*		ns
PN	ns	0.883*	0.873*	0.909**	0.976**	ns	0.894*	0.984**	ns	0.960**	0.893*	

SOC = soil organic carbon, TN = soil total nitrogen, NH_4^+-N = ammonium nitrogen, NO_3^--N = nitrate nitrogen, PN = plant nitrogen content, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, LAP = Leucine aminopeptidase, NAG = β-1, 4-N-acetylglucosaminidase. The values in the blue area are in the wet-hot season and in the yellow areas are in the dry-cool season. ns, * and ** = F-value not significant, significant at $P > 0.05$ and $P < 0.05$ and $P < 0.01$, respectively.

Pathway analysis for Eucalyptus to enhance N absorption

The structural equation model on the regulatory pathway of soil N dynamics well passed all the statistical tests on adequacy of microbial biomass and enzyme activities (Fig. 4a and 4b). Overall, soil pH, SWC, SOC and TN in both seasons were significantly higher at the MP than PP sites, which together stimulated microbial biomass and most of soil N-related enzyme activities although the control mechanisms are different between seasons. The enhanced microbial activity likely accelerated soil N mineralization, providing more available N (NH_4^+-N and NO_3^--N) for *Eucalyptus* absorption. Path analysis pointed to direct and positive controls of soil N availability (NH_4^+-N and NO_3^--N) by the activity of soil N-related enzymes in both seasons but the control by microbial biomass in the wet-hot season only. Soil environment positively corrected with microbial biomass in the dry-cold season only. SOC and TN positively corrected with both microbial biomass and enzyme activity in the dry-cold season and there was also a positive relationship between microbial biomass and enzyme activity. In the wet-hot season, SOC and TN did not directly affect soil enzyme activity, but have an indirect effect via influencing microbial biomass.

Discussion

Mixed plantations of *Eucalyptus* and N-fixing species have been recognized as one of effective silvicultural practices to increase *Eucalyptus* productivity while maintaining soil fertility, compared to *Eucalyptus* monocultures (Epron et al. 2013; Tchichelle et al. 2017). For example, introducting *Acacia mangium* into *E. grandis* provides better conditions for restoring the soil fertility and soil biodiversity, hence providing better sustainability of the cropping systems than pure *E. grandis* plantations (Garay et al. 2004). However, the results reported previously remain contradictory depending on the selected N-fixing species and stand conditions (Forrester et al. 2006; Firn et al. 2007; Bouillet et al. 2013). In this study, we discovered that the N absorption and productivity of *Eucalyputs* as well as soil TN and N availability were significantly enhanced by introducting *D. odorifera* into the *Eucalyptus* plantations. This suggests that mixed plantations of *Eucalyptus* with *D. odorifera* could be considered as an effective sustainable management option in Guangxi Zhuang Autonomous Region, southern China.

The enhanced N absorption on *Eucalyputs* with introduction of *D. odorifera* was likely attributed to the improved soil N availability, as N accumulation of *Eucalyputs* was directly correlated with both soil NH_4^+-N and NO_3^--N concentrations (Table 2). Using structural equation model, we found that the higher soil organic matter (SOC and TN) and improved soil environment (pH and SWC) could indirectly enhance soil N availability via stimulating microbial activity (microbial biomass and enzyme activity) (Fig. 4). Increased soil N storage under the mixed plantation of *Eucalyptus* and N_2-fixing species is common, as the growth of N-fixing plants relies largely on the fixed atmospheric-N (Paula et al. 2018). Therefore, introduction of N-fixing species into *Eucalyptus* plantations would decrease the N uptake of trees from soil, in turn increasing soil N retention (Bouillet et al. 2008). Faster growth of N-fixing tree species and *Eucalyptus* due to higher N availability at the MP sites could also increase the input of plant residuals and hence enhance SOC and TN. Similar results were also found in other studies with a mixed plantation such as *E. globulus* and *A. mearnsii* in Australia (Forrester et al. 2005), *Eucalyptus grandis and Acacia mangium* in Brazil (Bini et al. 2013a, 2013b) and in Congo (Koutika et al. 2019, 2020), or *Eucalyptus regnans* and *Acacia dealbata* in southeastern Australia (Pfautsch et al. 2009). Taken together, the higher SOC ,TN and inorganic N at the MP sites (Table 1) indicate that mixed-species plantation not only improved soil N availability, aiming at preventing soil degradation; but also increased soil C sequestration, contributing to mitigating climate change.

The greater soil microbial biomass at the MP than PP sites (Fig. 2) suggests that the presence of leguminous trees in the system could improve stand conditions for microbial proliferation (Bini et al. 2013a), while the driving factors differed between the seasons in this study. In the dry-cold season, soil microorganisms are usually subject to water limitation. Thus, increased SWC under the MP site could stimulate microbial biomass. This was supported by the positive relationship between MBC and SWC in the dry-cold season observed in this study (Table 2). The positive relationship between TN and MBN in the dry-cold season (Table 2), suggests that increased TN under the MP site could alleviate microbial N limitation and enhance microbial N assimilation. This in turn may explain why enhanced microbial biomass under the MP site did not directly contribute to higher N availability in the dry-cold season. In the wet-hot season, both temperature and SWC are relatively higher at our study sites, thus increased SWC and pH under the MP site did not significantly influence microbial biomass (Table 2). Alternatively, we found direct and positive effects of SOC and TN on microbial biomass, suggesting that microbial biomass in the wet-hot season was mainly limited by substrate availability in this study. Introduction of *D. odorifera* into *Eucalyputs* plantation significantly increased MBC but not MBN in the wet-hot season, thus having a direct and strong contribution to soil N availability. This was supported by the positive relationships of MBC with NH_4^+-N or NO_3^--N in the wet-hot season observed in this study (Table 2).

Microbial communities involve soil biogeochemical cycling primarily through producing specific extracellular enzyme (Bowles et al. 2014). Increased microbial biomass usually enhanced N-degrading extracellular enzyme production in the soil. Increased TN could also stimulate the activity of N-degrading enzymes as providing more N resource for microbial decomposition. We have observed positive relationships of MBC with the activities of all N-degrading extracellular enzymes in the wet-hot season, while in the dry-cold season the enzyme activity was controlled by both TN and microbial biomass (Fig. 4). Previous studies have also reported that hydrolytic enzyme activities increased (Huang et al. 2017) with increasing soil TN and MBC since N-fixing trees were introduced into the *Eucalyputs* plantations (Bini et al. 2013b; Huang et al. 2017). It is well known that increasing in N-degrading enzyme activities reflect a higher rate of N mineralization and a higher level of N availability (Tabatabai et al. 2010). Although we have not determined the N mineral processes in this study, previous studies have frequently found that introducing N-fixing trees into *Eucalyptus* plantations can stimulate microbial activity (Pereira et al. 2018, 2019) to increase the rate of N mineralization (Voigtlaender et al. 2012, 2019). Alternatively, we indeed found NH_4^+-N was positively correlated with the N accumulation of *Eucalyptus* in both seasons, while the similar relationship was only occurs in the wet-hot season for NO_3^--N. This result is consistent with the observation that the growth of many forest plant species depend on ammonium (Eiter 1972).

Conclusion

Compared with the *Eucalyptus* monoculture, we found that the N absorption and productivity of *Eucalyptus* were significantly increased by introduction of *D. odorifera* into the *Eucalyptus* plantation. Introduction of *D. odorifera* into the *Eucalyptus* plantation could also improve soil N availability and increase soil C and N storage. Moreover, our results highlight the importance of microbially mediated soil N cycling in the N absorption on *Eucalyputs*. These findings in turn can provide government and policy makers with useful tools to achieve N sustainable management in *Eucalyptus* plantations.

Abbreviations

TN: total nitrogen, SOC: soil organic carbon, SWC: soil water content, PP: pure *E. urophylla* × *grandis* plantation, MP: a mixed species plantation of *D. odorifera* and *E. urophylla* × *grandis*, MBC: soil microbial biomass carbon, MBN: soil microbial biomass nitrogen, LAP: leucine aminopeptidase, NAG: β-1,4-N-acetylglucosaminidase.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data available on request from the authors.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31460196 and 31870461), the Innovation Project of Guangxi Graduate Education (NO. YCBZ2018012), the “Hundred Talent Program” of South China Botanical Garden at the Chinese Academy of Sciences (No. Y761031001), and the “Young Top-notch Talent” in Pearl River talent plan of Guangdong Province, and by the Science (No. 2019QN01L763).

Acknowledgements

All who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials have acknowledged.

The authors are grateful to Junfei Xiong at Chinese Academy of Forestry, Guangxi, China, for collecting the soils, harvesting and measuring of the plants in the field. We are also grateful to Dafeng Hui at Tennessee State University, Nashville, TN, USA, for his valuable comments and English improvements.

Authors’ contributions

All the authors designed the experiments; Xianyu Yao, Zhi Nong and Qianchun Zhang carried out the experiments; Xianyu Yao, Shaoming Ye and Qi Deng analyzed the experimental results, Xianyu Yao and Haiju Zhou analyzed the data and developed analysis tools; Xianyu Yao, Shaoming Ye and Qi Deng wrote the manuscript, and all authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

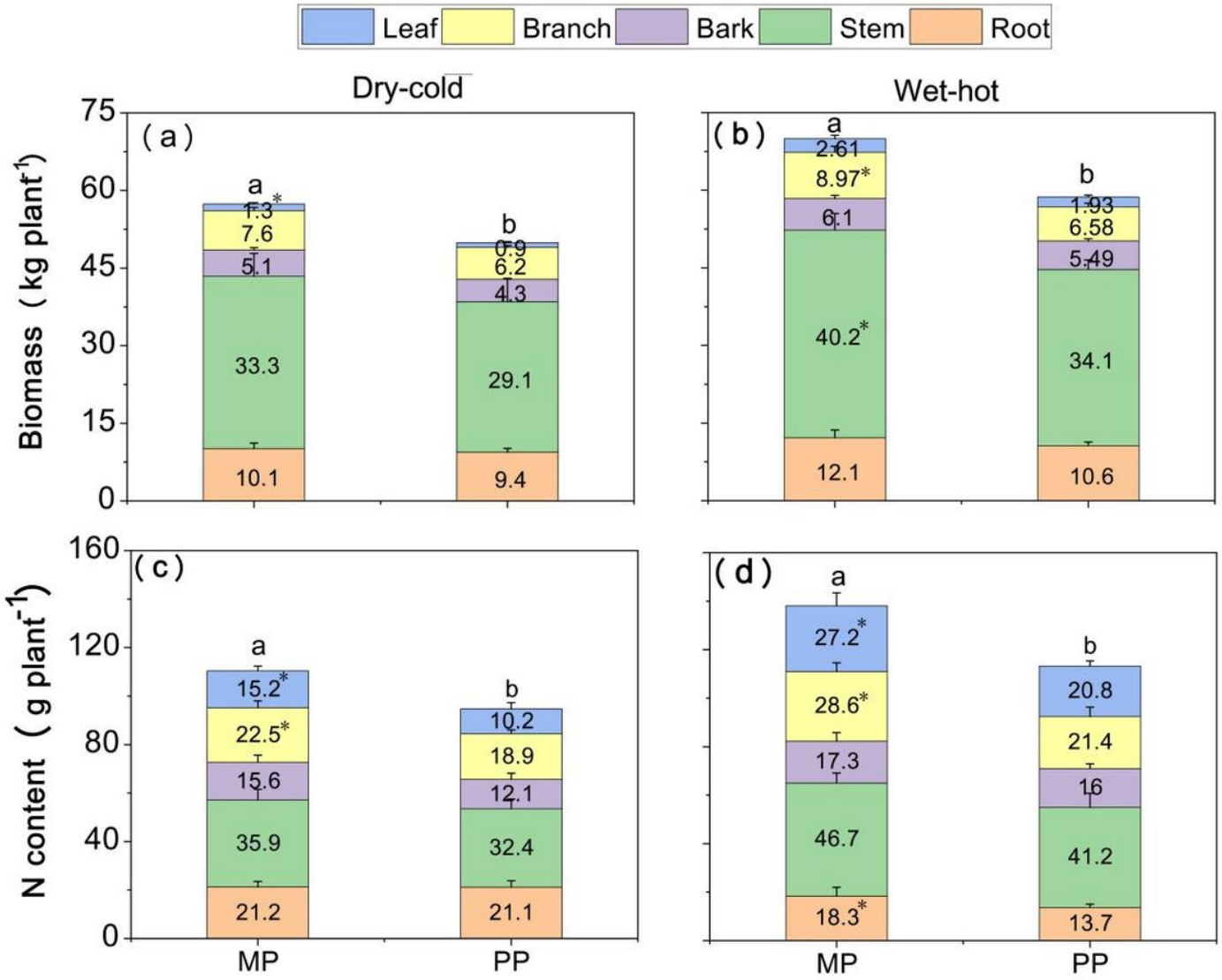

Figure 1

The biomass and the N accumulation of E. urophylla × grandis on pure Eucalyptus and mixed with N2-fixing species and Eucalyptus, respectively. (a), (b) represent the biomass of E. urophylla × grandis and (c), (d) represent the N content of E. urophylla × grandis in dry-cold season and wet-hot season, respectively. PP = pure Eucalyptus plantations; MP = mixed with N2-fixing species Eucalyptus plantations. Different lowercase letters (a and b) on the barks are different significantly of the whole plant ($p < 0.05$) and * is different significantly in the same organ between the PP and MP site.

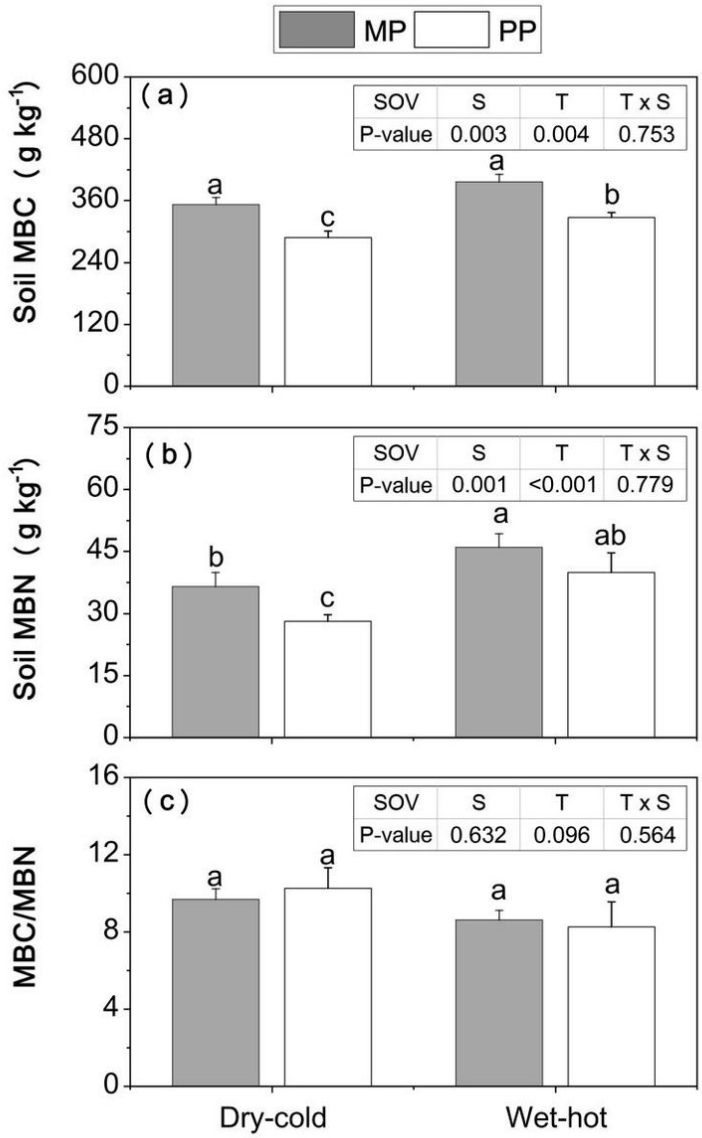

Figure 2

The soil microbial biomass carbon (MBC) (a), microbial biomass nitrogen (MBN) (b) and MBC:MBN ratio (c) in the pure Eucalyptus plantations and mixed with N2-fixing species and Eucalyptus plantations in both dry-cold and wet-hot seasons. PP = pure Eucalyptus plantations; MP = mixed with N2-fixing species Eucalyptus plantations. Different lowercase letters (a, b and c) on the barks indicate significant differences under different treatments at $p < 0.05$. Error bars indicate ± SE (n = 3). For the table in the top right-hand corner of figure, 'SOV', 'S', 'T' and 'S × T' indicate that 'Source of variation', 'season' 'stand type' and 'interaction season × stand type', P-values show that was not significant and significant affected for $p>0.05$ and $p<0.05$.

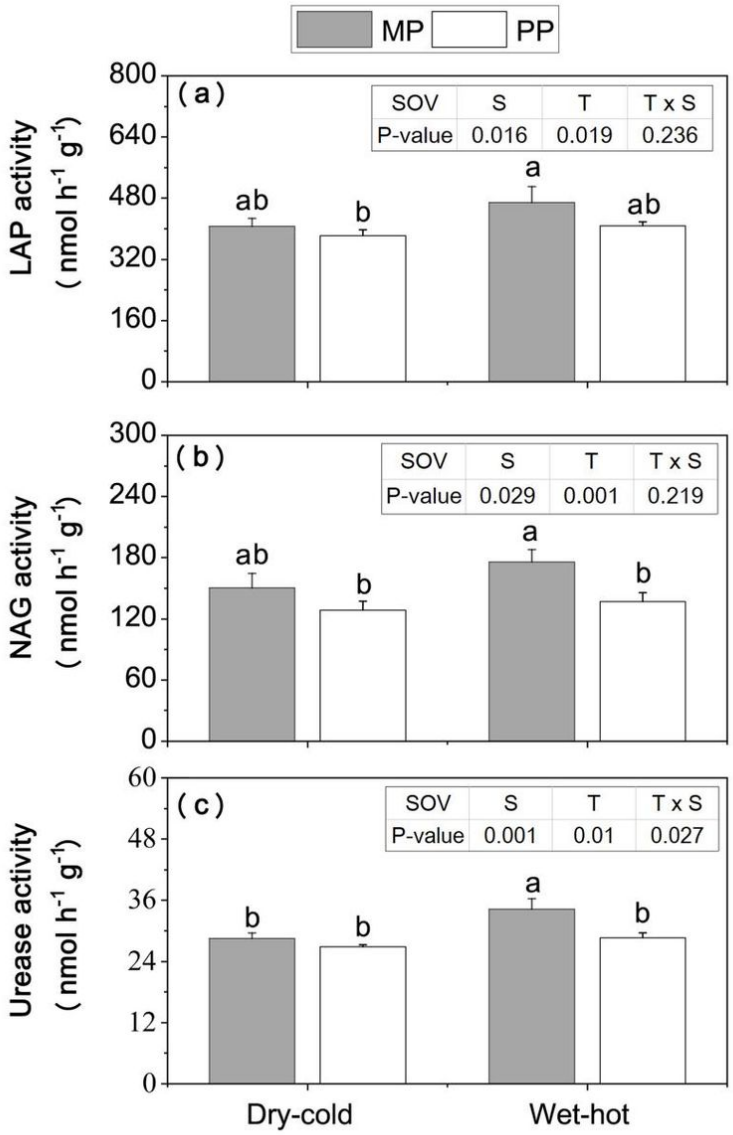

Figure 3

The soil enzyme activities per gram of soil in the pure Eucalyptus plantations and mixed with N2-fixing species and Eucalyptus plantations in both dry-cold and wet-hot seasons. PP = pure Eucalyptus plantations; MP = mixed with N2-fixing species Eucalyptus plantations. Different lowercase letters (a and b) on the barks indicate significant differences under different treatments at $p < 0.05$. Error bars indicate ± SE (n = 3). For the table in the top right-hand corner of figure, 'SOV', 'S', 'T' and 'S × T' indicate that 'Source of variation', 'season' 'stand type' and 'interaction season × stand type', P-values show that was not significant and significant affected for $p>0.05$ and $p<0.05$.

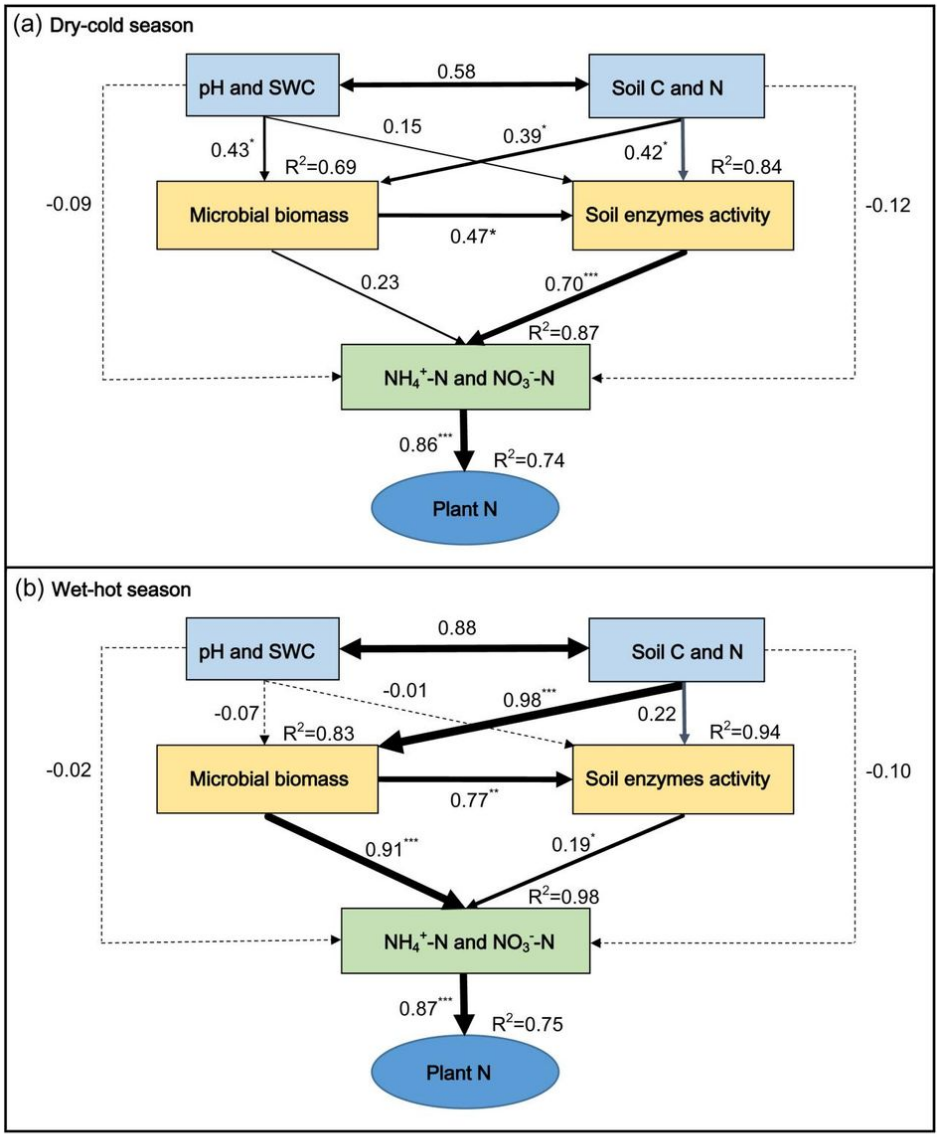

Figure 4

Path model depicting the regulatory pathway of the controls of soil extracellular enzymes activities of soil physical-chemical properties (pH and soil water content: SWC), total nitrogen (TN), soil organic carbon (SOC) and microbial biomass (MBC and MBN) by the structural attributes to involve plant N absorption by plant. Where (a) represent structural equation model (SEM) in the dry-cold season for the model were x2=17.318, p=0.002, CMIN/df=4.330, GFI=0.897, RMSEA=0.380, CFI=0.913; and (b) represent SEM in the wet-hot season for the model were x2=11.308, p=0.023, CMIN/df=2.827, GFI=0.962, RMSEA=0.276, CFI=0.974. The black solid lines and dotted lines indicate significant positive and negative relationships, respectively; the thickness of the arrows reflect the degree of relationships, numbers at arrows are standardized path coefficients, and R2 values indicate the variation of response variables explained by the model. *, ** and *** are different significantly at $p < 0.05$, $p<0.01$ and $p < 0.001$, respectively.

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