Original Research Article

Rhizosphere effects of *Melocannabaccifera* on soil microbial properties under different fallow phase following shifting cultivation

ABSTRACT

Rhizosphere plays an important role in regulating soil fertility and nutrient cycling in different ecosystems. Bamboos are important secondary successional plants in fallow landthat have strong impact on the soil fertility of different fallow lands. In this paper, we examined the rhizosphere effects of bamboo(*Melocannabaccifera*) on soil microbial properties (soil organic carbon, SOC; total nitrogen, TN; microbial biomass C and N, MBC and MBN; dehydrogenase activity, DHA; acid phosphatase activity, APA; β-glucosidase activity, GSA) in rhizosphere (RS) and bulk soil (BS) in shifting cultivation stand with different fallow phase (2 years old, FP-2; 5 years old, FP-5; and 10 years old fallow, FP-10) in Mizoram. The result indicates that soil microbial properties were significantly higher(p<0.05) in RS compared to BS. Further, the level of microbial properties was significantly greater in longer fallow (FP-10) compared to shorter fallow (FP-2 and FP-5). On contrary, magnitude of rhizosphere effect of *M. baccifera* was greater short fallow phase compared to longer fallow for all microbial properties except in APA and GSA. In addition, microbial properties were significantly high (p<0.05) in RS compared to BS. It is concluded that the rhizosphere effect of bamboo in shorter fallow is microbial mediated under C and nutrient limited conditions and in longer fallow the same is regulated by the accumulated organic matter and the available nutrients. Further studies are needed to assess the changes in secondary successional plant rhizosphere microbes under different fallow phases.

Keywords: Rhizosphere soil; bulk soil; fallow land; microbial properties; enzyme activities.

1. Introduction

Since, Hiltner coined the term “rhizosphere”, a narrow zone around the roots, in 1904 and observed enhanced diversity of microorganisms in this zone compared to the bulk soil (BS), and emphasized the potential importance of microbial activities associated with root systems in plant nutrition. Majority of published studies on rhizosphere soil (RS) nutrient cycling for trees were conducted on seedlings in microcosms and only few field studies in forests trees are available [1,2]. The rhizosphere nutrient cycling of trees under field conditions may vary greatly from that of annual plants and tree seedlings due to their differences in nutrient requirement, soil conditions, as well as growth period [2]. Recently, tree rhizosphere has been reported to greatly affect the global cycling of carbon and nutrients in a changing environment [3]. Therefore, studies on rhizosphere effects of different plants under field condition attract attention of the scientists over the world where the information is highly limited.

The term ‘rhizosphere’ is now used in a more general sense to describe the effect of different root systems on soil physical and chemical properties [4]. This region has been reported as crucial importance for plant health and nutrition [5] and thus rhizosphere microbial processes are important for vegetation development and reestablishment during the process of recovery. Number of researchers have investigated the difference between the microbial communities of RS and BS using phospholipid fatty acid profiles [6,7,8,9] or molecular techniques [10]. The results of these investigations suggested that the differences in rhizosphere microbial community among plant species are largely attributed to the different root exudations. Garcia et al. [11] reported higher microbial biomass in rhizosphere associated with *Stipatenacissima* and *Retamasphaerocarpa* species and recommended these species for soil restoration in semiarid conditions.

Shifting cultivation is an old age practice of agriculture predominant in Northeast India. Mizoram is one of the seven sister states of northeast India where majority of the population (~60%) depends on agricultural products from shifting cultivation carried out on steep slopes (~half of the total land area is having 40-100% slopes). This makes the region different from other northeastern states to perform many activities (i.e. slashing, burning, sowing, weeding and harvesting) on these steep slopes, and is responsible for huge loss (~60 t ha^{-1}) of fertile soils every year through erosion [12]. Earlier, the practice was adequately productive, economically feasible and ecologically balanced because of prolonged fallow period (~20-30 years) but in recent years as a result of exponential expansion of human population, fallow periods have been drastically reduced (~<5 years) which has led to substantial decrease is soil fertility and crop productivity [13]. Singh et al. [14] recommended about 10-15 years of minimum fallow periods to maintain the soil fertility, particularly C and N, for sustainable crop production, which are the key factors for the plant growth in the region. Bamboo forest accounts for about 57% of the total forest area of the state. *M.bacciferais* one of the most dominant species of bamboo and occupies 95% of the total bamboo forest. *M. baccifera* is an efficient early colonizer secondary successional species characterized by a woody leptomorph rhizome system [15] that spreads quickly to recover the land after shifting cultivation [16]. Several studies emphasized the role of bamboos in stabilizing nutrient cycling in the early successional fallows of slash and burn agriculture systems of northeast India [17].

Changes to the microbial properties of rhizosphere soil have significant influence on the sub sequential growth and health of plants. *M. baccifera* has been reported to have significant changes on soil fertility following shifting agriculture in subtropical forests [17,18]. Therefore, it is important to understand the magnitude of rhizosphere effect of bamboo (*M. baccifera*) on soil microbial properties. The main objectives of the present studies are i) to determine changes in soil microbial properties between *M. baccifera* RS and BS ii) to

understand variation in RS microbial properties in different fallow phases following shifting cultivation in Mizoram. We hypothesize that the rhizosphere effect of *M. baccifera* in shorter fallow phase has greater microbial changes than the longer fallow phase.

2. Materials and methods

2.1 Site description and soil sampling

The study was conducted at three different fallow phase (2, 5 and 10 years) in Muallungthu and Tachhip villages in Aizawl district of Mizoram. The 2 years fallow phase (FP-2) is located in Muallungthu (23°36.305' N and 92°42.873' E) at 838m altitude and the other two sites 5 years fallow phase (FP-5) and 10 years fallow phase (FP-10) are located in Tachhip village (23°35.699' N and 92°43.096' E at 740m altitude) and (23°35.667' N and 92°43.081' E and at 725m altitude) respectively. The ages of fallow lands were identified through interviewing the land owner. The soil of the study sites belongs to order inceptisol and falls under red soil group. Soil is light to medium texture (sandy loam and clay loam) and slope of the land varied between ~35º and 40º. The mean minimum and maximum temperature of the study sites ranged from 11-21°C and 20- 30°C respectively. The annual average rainfall of the study area is 2350 mm. Soil bulk density ranges from 0.94-1.1 g cm^{-3}.

Soils were sampled from the upper 20 cm depth by excavating 4 soil cores (5 cm diameter) from 5 random blocks (5m x 5m) in June, 2013. Twenty soil cores (5 blocks × 4 soil cores = 20 soil cores) were pooled together to have one composite sample of approximately 500g from each site. RS was collected by gentle shaking followed by use of a forceps to remove the soil from the live roots and the remaining was considered as BS. Each composite soil was divided into four replicates and the replicated samples were divided into 2 parts: one part was placed in ziplock bag and kept in freezer at -20°C as fresh sample for analysis of microbial

properties and the other part was air dried in the laboratory. Microbial biomass and enzyme activities were analyzed before two weeks to avoid alteration of microbes due to freezing.

2.2 Laboratory analysis

Gravimetric soil moisture content (%) was estimated by oven drying the known weight of field moist soil. Air dried soil (passed through 0.5mm sieve) were used to analyzed soil pH, soil organic carbon (SOC) and total nitrogen (TN). Fresh soil samples (passed through 1mm sieve) were used to analyze microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), acid phosphatase activity (APA), β-glucosidae activity (GSA) and dehydrogenase activity (DHA). Soil pH was measured with a glass electrode (1:2.5 soils: water ratio). SOC was determined by the K2Cr2O7 wet-oxidation method [19] and TN was analyzed using CHN analyzer (CHNS-O Elemental Analyzer EUROEA, 3000). For determination of soil MBC and MBN, fresh soil samples (25 g) were subjected to the chloroform–fumigation–extraction method [20]. The difference between fumigated and non-fumigated samples in terms of C, N, and P was determined and then, MBC, MBN and MBP were calculated using conversion factors, K_{EC} = 0.38, K_{EN} = 0.45 respectively [21,22]. The APA was determine by method described by Tabatabai[23] and was expressed as mg pNP kg^{-1}dw soil h^{-1}, DHA by Casida et al. [24] and expressed as mg TPF kg^{-1}dw soil h^{-1} whereas GSA was determined by Eivazi and Tabatabai[25] method and expressed as mg pNG kg^{-1} dw soil h^{-1}.

2.3 Statistical Analysis

All statistical analysis like ANOVA, Kruskal-Wallis H test, paired sampled *t*-test, principal component analysis (PCA) was performed in software package IBM SPSS Statistics 20.0 for Windows. Paired sample *t*-test was performed to test the significant differences in soil variables between RS and BS. The three fallow phases were compared in terms of every parameter analyzed by the non-parametric Kruskal–Wallis H test, incorporating 1000

randomizations at a Monte-Carlo test of significance with a 99% confidence limit. One-way analysis of variance (Tukey's HSD) was performed to test the rhizosphere effects on soil microbial properties of different fallow phase. The magnitude of the rhizosphere effect was calculated as the percentage difference between paired RS and BS samples for each soil variable.

3. Results

3.1 Soil physico-chemical and microbial properties between rhizosphere and bulk soil

The SMC in RS was significantly higher ($p<0.05$) than BS but soil pH showed significant decreased in RS compared to BS in all the sites (Table 1). Similarly, amount of SOC and TN in RS was significantly higher ($p<0.05$) compared to BS for all the sites except in FP-2, where no significant changed in TN between RS and BS (Table 1). The MBC and MBN values were significantly higher ($p<0.05$) in RS compared to BS far all the sites (Table 2). Correspondingly, the value of APA showed significant variation between RS and BS, however, no significant changed between RS and BS in DHA for all the sites (Table 2). The value of GSA marked significant variation between RS and BS in FP-2 and FP-10 but no significant variation in FP-5 (Table 2).

Table 1

Soil moisture content (SMC), soil pH, soil organic carbon (SOC) and total nitrogen (TN) in rhizosphere soil (RS) and bulk soil (BS) of different fallow phases, Mizoram.

Soil	Fallow phase	MC (%)	pH	SOC (%)	TN (%)
Rhizosphere soil	2 years	23.6(4.8)[a]	4.46(0.3)[a]	2.8(0.1)[a]	0.27(0.01)[a]
	5 years	25.1(5.7)[a]	4.71(0.2)[a]	2.9(0.1)[a]	0.29(0.02)[a]
	10 years	27.2(5.3)[a]	4.98(0.3)[a]	3.1(0.1)[a]	0.30(0.02)[a]
	Monte Carlo Sig.	0.044	0.037	0.042	0.200
Bulk soil	2 years	20.7(4.3)[b]	4.23(0.2)[b]	2.1(0.1)[b]	0.26(0.01)[a]
	5 years	21.9(4.9)[b]	4.56(0.4)[b]	2.3(0.2)[b]	0.26(0.05)[b]
	10 years	22.8(5.7)[b]	4.96(0.4)[a]	2.6(0.2)[b]	0.27(0.05)[b]
	Monte Carlo Sig.	0.314	0.037	0.124	0.229

Values are means of 4 replicates ± standard deviation. From each fallow phase, differences in soil variable between RS and BS were determined by paired sample *t*-test. Same letters indicate non-significant difference between RS and BS. Variation in soil parameters in different fallow phase were determined by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limits.

Table 2

Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), acid phosphatase activity (APA), β-glucosidase activity (GSA) and dehydrogenase activity (DHA) in rhizosphere soil (RS) and bulk soil (BS) of different fallow phases, Mizoram.

Soil	Fallow phase	MBC (µg g^{-1})	MBN (µg g^{-1})	APA (µg PNP g^{-1} soil h^{-1})	GSA (µg PNG g^{-1} soil h^{-1})	DHA (µg TPFg^{-1} soil h^{-1})
Rhizosphere Soil	2 years	469(69)^{a}	36(7)^{a}	425(55)^{a}	29.5(1.1)^{a}	5.4 (1.7)^{a}
	5 years	503(74)^{a}	39(9)^{a}	628(68)^{a}	29.6(0.5)^{a}	6.7(2.0)^{a}
	10 years	551(69)^{a}	46(12)^{a}	1462(85)^{a}	39.9(1.1)^{a}	6.8(2.7)^{a}
	Monte Carlo Sig.	0.001	0.438	0.014	0.015	0.668
Bulk Soil	2 years	349(46)^{b}	17(5)^{b}	337(20)^{b}	25.2(0.3)^{b}	4.6(0.1)^{a}
	5 years	401(63)^{b}	26(8)^{b}	347(70)^{b}	26.9(2.4)^{a}	5.5(0.7)^{a}
	10 years	443(54)^{b}	34(9)^{b}	648(18)^{b}	27.2(2.4)^{b}	5.9(1.5)^{a}
	Monte Carlo Sig.	0.001	0.017	0.006	0.749	0.265

Values are means of 4 replicates ± standard deviation. From same fallow phase, differences in soil variable between RS and BS were determined by paired sample *t*-test. Same letters indicate non-significant difference between RS and BS. Variation in soil parameters in different fallow phase were determined by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limits.

3.2 Variability in soil variables as influenced by fallow phase

The three fallow phases (FP-2, FP-5 and FP-10 years) showed significant variation in terms of SMC, pH, SOC, MBC, APA and GSA for RS but no significant variation in TN, MBN and DHA, on the other hand, significant variation was marked in soil pH, MBC, MBN and APA whereas no significant changed in SMC, SOC, TN, GSA and DHA for BS (P>0.05; Kruskal–Wallis H test incorporating Monte-Carlo significance at 99% confidence limit; Tables 1 and 2). The overall variability of different fallow phase in RS and BS were presented in Fig.1 a

and b respectively. PCA generated two distinct clusters with FP-2 and FP-5 separated from FP-10 in RS with PC1 (50.41%) and PC2 (21.87%) and BS with PC1 (65.36%) and PC2 (17.37%) (Fig.1 a and b). The magnitude of rhizosphere effect of *M. baccifera* in different fallow period was listed in Table 3. The results showed that rhizosphere effect was greater in 2 years fallow for different soil variables except in APA and GSA. The rhizosphere effect in SOC ranged from 40-89%, TN (5-12%), MBC (24-34%), MBN (34-111%), APA (56-126%), GSA (17-48) whereas no rhizosphere effect was marked in DHA. Correlation analyses (data not given) showed that SOC positively significantly correlated with MBC (r^2=0.447, P<0.009), MBN (r^2=0.392, P<0.032), APA (r^2=402, P<0.002) but negatively correlated with soil pH (r^2= -0.419, P<0.05) whereas TN positively significantly correlated with MBC (r^2=0.730, P<0.001), MBN (r^2=0.590, P<0.001), APA (r^2=0.741, P<0.001), GSA (r^2=0.699, P<0.002) but negatively correlated with soil pH (r^2= -0.527, P<0.527) and SMC (r^2= -0.149, P<0.075).

Table 3

Magnitudes of rhizosphere effects (%) of bamboo (*M. baccifera*) on soil in different fallow land (n=4) following shifting cultivation in Mizoram.

Fallow	SOC	TN	MBC	MBN	APA	GSA	DHA
2 years	82^a	5^b	34^a	111^a	56^c	17^b	NS
5 years	59^b	11^a	25^b	45^b	81^b	NS^c	NS
10 years	40^c	12^a	24^b	34^b	126^a	48^a	NS

Values in columns with different superscript letters denote significant differences between fallow land at P<0.05. NS shows no significant rhizosphere effect. Soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), acid phosphatase activity (APA), dehydrogenase activity (DHA), β–glucosidase activity (GSA).

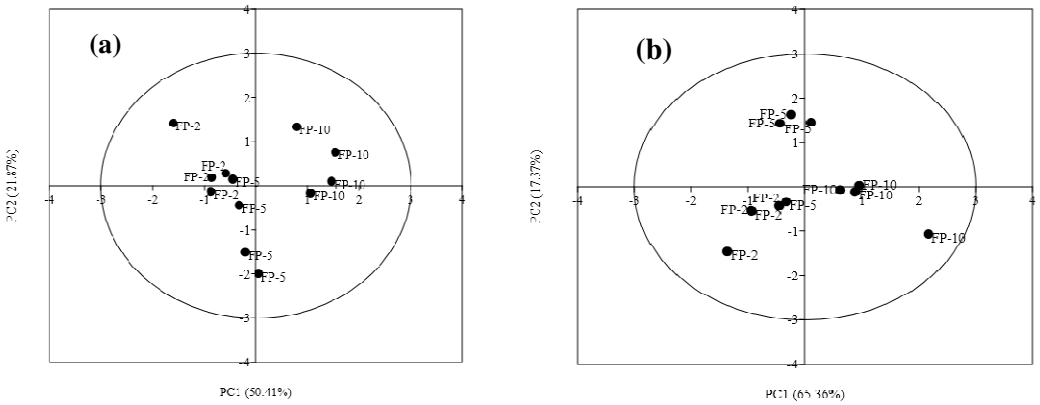

Fig.1. Scores of the first two principal components of microbial properties in (a) rhizosphere soil (b) bulk soil of different fallow phase e.g. 2 years fallow phase (FP-2), 5 years fallow phase (FP-5) and 10 years fallow phase (FP-10) in Mizoram.

4. Discussion

4.1 SOC, TN and pH in rhizosphere and bulk soil

The rhizosphere is a narrow zone with high microbial activity and turnover rates driven by plant root exudates, making its chemical and microbial properties usually distinct from those of BS [9]. Root exudates affect nutrient cycling by stimulating rhizosphere microbial growth and altering their community structure [26]. In the current study, the RS of *M. baccifera* showed higher SOC and TN than BS. The result of increase SOC and TN in RS may be due to rhizodeposition, which may account for as much as 25% of the C allocated belowground (or 10% of the net fixed C and N) [27,28]. The change in rhizosphere pH has been demonstrated by several researchers [2,29,30]. The changes in soil pH in the present study were similar to that in rhizosphere of silver birch by Rosenvald et al. [31] and Zhang et al. [29]. Root exudation of organic anions as well as respiration in alkaline soils, cation-anion exchange balance by roots, and redox-coupled processes may be responsible for lower soil pH in RS.

4.2 Microbial properties in rhizosphere and bulk soil

The current study shows that RS of *M. baccifera* have higher MBC and MBN contents compared to BS. A similar trend was marked in enzyme activities like APA and GSA except in DHA. The results in present studies are in consistent with those of earlier studies [7,32]. These results suggest that the rhizosphere microbes are more dynamic and active than those in the BS. Enhanced microbial properties in RS may be due to root exudation such as sugars, acids, hormones, sloughed root cells and C allocated to root-associated symbionts. These substrates provide favorable resources for the microbial population. By contrast, there is no significant variation in DHA between RS and BS. This finding is in contrast to the results reported by Nosalewicz and Nosalewicz[33]. The non-significant variation in DHA between RS and BS may be related to the compositions of microbial communities in rhizosphere. Buee et al. [34] and Koranda et al. [9] described that soil enzyme activities are formed by some specialized groups ofmicroorganisms through an r or K strategy, e.g. phosphatase produced by mycorrhizal fungi. These microorganisms have different capacities to assimilate plant derived C sources.

4.3 Rhizosphere effects of *M. baccifera* in different fallow phase

The amount of organic matter buildup during the course of succession in moist tropical forest of Mizoram is originally influenced by the length of fallow phase and thus the stands with higher abandoned age supports greater crop productivity [13]. *M. bacciferra* is a secondary successional species that recover quickly during the course of succession and often regenerate through rhizome during the cropping phase and interact with crops. The fallow phase can significantly influence the microbial properties in the plant rhizosphere. In the current investigation, the amount of SOC and TN in rhizosphere was significantly increased in longer fallow phase. These results indicate that the RS of *M. baccifera* in long fallow phase has greater potential to enhance SOC and TN compared to short fallow phase. Similarly, the

amount of MBC and MBN significantly enhance with increase fallow phase demonstrating increased microbial activities on nutrient substrates. A similar result was observed by Rosenvald et al. [31] in their investigation of the rhizosphere soil microbial properties in a chronosequence of silver birch stands on reclaimed post-mining areas.Similarly, the amount of APA and GSA increased considerably from shorter fallow to longer fallow phase. A possible reason for decline in enzyme activities in short fallow phase may be the result of nutrient limitations whereas in longer fallow phase, adequate supply of nutrient substrate through organic matter decomposition may enhance the rate of microbial activity. The findings in current investigation is consistent with the result reported in rhizosphere microbial properties of successional annual plants (*Artemisia capillaries* and *A. sacrorum*) in semi-arid region of Loess plateau, China [29]. Tarafdar and Jungk[35] carried out a very interesting study on the relationship between soil enzyme activity and nutrient cycling in the rhizosphere and their result demonstrated that enzyme activities increased with plant age, probably due to increase in microbial biomass and/or the increase in total root surface area. Furthermore, the low nutrient availability in the short fallow phase possibly inhibits the microbial utilization of C released by the roots. However, in the longer fallow phase, increased soil nutrients fueled microbial growth and activity [36,37].

The result in present finding indicates increase microbial properties with fallow phase. On contrary to this result, the magnitude of rhizosphere effect of *M. baccifera* is greater in shorter fallow phase than longer fallow phase which is in conformity with our hypothesis. The greater rhizosphere effect in short fallow phase may be due to increase root exudation of *M. baccifera*as well as microbial activity in the rhizosphere that tends to build up and sustain nutrient level mainly in low nutrient availability. The improvement of nutrient substrate in BS may also be responsible for reducing the rhizosphere effect in longer fallow phase. Earlier studies reported that the magnitude of rhizosphere effect of Sugar maple and Red oak on

biochemical properties were affected by fertilization and their results indicated that rhizosphere effect was greater in control plot (without fertilization) compared with fertilized plots [1]. This result shows that the magnitude of rhizosphere effect of plants is largely affected by the soil condition relatively than the host plant. Singh et al. [38] reported that the major factor causing changes in microbial composition in rhizospheres is soil, rather than plants.

5. Conclusion

The present study shows that the bamboo rhizosphere has significant changes in soil microbial properties in different fallows with greater effect in short fallow phase. It appears that rhizosphere effect of bamboo in young fallow is driven by microbes growing under C and nutrient limited conditions and the same in older fallows are regulated by the opportunistic soil microbes by exploiting the organic matter and the nutrients accumulated there. Further studies are needed to assess the changes in rhizosphere microbes in different fallow phase.

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