

Physico-chemical, biochemical and microbial properties of the rhizospheric soils of tree species used as supports for black pepper cultivation in the humid tropics

R. Dinesh ^{a,*}, V. Srinivasan ^a, S. Hamza ^a, V.A. Parthasarathy ^a, K.C. Aipe ^b

^a Indian Institute of Spices Research, P. O. Box 1701, Marikunnu P.O., Calicut-673012, Kerala, India

^b Banana Research Station, Kerala Agricultural University, Kannara-680652, Kerala, India

ABSTRACT

The most popular live stakes used as supports in black pepper (*Piper nigrum* L.) plantations of the humid tropics are *Ailanthus triphysa* (Dennst.) Alston., *Erythrina variegata* L., *Gliricidia sepium* (Jacq.) Steud. and *Garuga pinnata* Roxb. Studies on soil properties in the rhizosphere of these tree species are limited. We report here information on soil physico-chemical, biochemical and microbial properties in the rhizosphere of these tropical trees. A non-living support (granite pole) was also included in the study as control. Among the tree species, greater levels of soil organic C were registered in the rhizosphere of *G. sepium* (26.5 g kg⁻¹), while the lowest level was registered by *A. triphysa* (21.6 g kg⁻¹). Greater levels of dissolved organic-C, -N, and mineral N also corresponded to the rhizospheres of *G. pinnata* and *G. sepium*, while the lowest level was registered by *E. variegata* and *A. triphysa*. Bray P level was markedly greater in the *G. pinnata* rhizosphere (8.4 mg kg⁻¹) and least in the *E. variegata* (5.2 mg kg⁻¹) rhizosphere. The levels of exchangeable-K, -Ca and -Mg in the tree rhizospheres were, however, not always higher than the control. Greater accumulation of microbial biomass-C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC}) was observed in the tree rhizospheres compared to the control. Among the tree species, C_{MIC} levels were greatest in the *G. sepium* and *G. pinnata* rhizospheres (474.0 and 454.0 µg g⁻¹ respectively) and least in the *E. variegata* rhizosphere (415.0 µg g⁻¹). However, N_{MIC} and P_{MIC} levels did not vary markedly among the tree rhizospheres. The C_{MIC}:SOC ratio was, in general, greatest for *A. triphysa* rhizosphere (1.96%) and least for the control (1.37%). In contrast, C_{MIC}:N_{MIC} ratio was greatest for control (18.6) compared to the tree species (14.3–15.1). The C_{MIC}:P_{MIC} ratio was greatest for *G. sepium* and *G. pinnata* (34.8 and 32.0 respectively) and almost identical for *A. triphysa*, *E. variegata* and control (28.8, 29.6 and 28.4 respectively). Soil respiration rates did not vary significantly among the tree species (22.0–25.0 µg CO₂-C g⁻¹ day⁻¹) and the lowest soil respiration (17.0 µg CO₂-C g⁻¹ day⁻¹) was registered in the control. Contrarily, metabolic quotient (qCO₂) was greatest for control (83.0 mg CO₂-C (g biomass C)⁻¹ day⁻¹) and least for the tree species (51.0–59.0 mg CO₂-C (g biomass C)⁻¹ day⁻¹). The tree rhizospheres also positively affected the activities of enzymes like dehydrogenase, urease, acid phosphatase, aryl sulphatase and β-glucosidase. Principal component analysis (PCA) reflected the strong relationship between microbial activity and the availability of labile and easily mineralisable organic matter, the logical dependence of microbial biomass on soil nutrients and a decrease in substrate use efficiency in soils with low organic substrates. The results imply that among the tree species studied, *G. sepium* and to some extent *G. pinnata* can be used as live supports for the restoration of degraded black pepper plantations and overall improvement in soil quality in the plains of the tropics.

Keywords:

Erythrina variegata

Garuga pinnata

Gliricidia sepium

Ailanthus triphysa

Piper nigrum

Rhizosphere soil properties

1. Introduction

Black pepper (*Piper nigrum* L.) is the most important spice traded internationally and is cultivated in many tropical regions of the world like India, Brazil, Vietnam, Indonesia, Malaysia and Sri Lanka. A variety

of trees species are used as live stakes for supporting black pepper. However, not all are considered ideal. Around 31 tree species which support the growth of black pepper have been identified under homestead agroforestry in Kerala, India (Salam et al., 1991). Among these, the most popular live stakes used on a plantation scale in the lower elevations are *Ailanthus triphysa* (Dennst.) Alston., *Erythrina variegata* L., *Gliricidia sepium* (Jacq.) Steud. and *Garuga pinnata* Roxb. (Nair, 1993). Also, non-living stakes made of granite or reinforced cement concrete are sometimes used to support black pepper. While silver oak (*Grevillea robusta* A.Cunn. ex R.Br) is the common support used in the higher elevations, *G. sepium* (Jacq.) Steud. and to some

* Corresponding author. Division of crop production, Indian Institute of Spices Research, P. O. Box 1701, Marikunnu P.O., Calicut-673012, Kerala, India. Tel.: +91 0495 2731410; fax: +91 0495 2730294.

E-mail address: rdinesh2005@gmail.com (R. Dinesh).

extent *E. variegata* L., are commonly used in Sri Lanka, Indonesia, Malaysia and the Philippines.

Major soil constraints on productivity of black pepper in the humid tropics include low nutrient reserves and P limitation due to fixation and Al toxicity (Srinivasan et al., 2007). Therefore, the role of support trees assumes great significance, because it should not only provide good physical support but should also sustain soil quality and benefit black pepper growth by positively influencing nutrient cycling in the rhizosphere. Since organic matter inputs to soil come primarily from plants via rhizodeposition and litter fall (Ayres et al., 2009), the tree species used as supports in black pepper plantations are expected to strongly influence the physical, chemical and biological properties of soils. A growing body of evidence has demonstrated that tree species can differ in their influence on soil properties (Augusto et al., 2002; Russell et al., 2007) and create distinctive soil environments and biotic communities. Hence, a better understanding of the mechanisms by which they influence soil properties, will improve our ability to predict the effect of tree species on the ecosystem. An enhanced predictive capacity has many applications, including restoration of degraded black pepper plantations, designing of sustainable black pepper-tree support systems, selection of tree species for C sequestration and improvement of soil quality in the tropics.

Distinct effects on soil processes of temperate and tropical tree species have been recorded and were attributed to litter quality, root exudates, and nutrient uptake (Augusto et al., 2002; Ilstedt et al., 2003). In addition to their effects on forest floors, the rhizospheric soil processes of the tree species are one of the very important factors in deciding their survival and sustainable growth. There have been numerous reports on rhizosphere soils under tree seedlings in mesocosms (Bradley and Fyles, 1995; Priha et al., 1999) and mature trees in temperate and tropical forests (Russell et al., 2007; Turpault et al., 2007; Phillips and Fahey, 2008). However, studies on soil properties in the rhizosphere of tree species used extensively as live supports in black pepper plantations of the tropics are lacking. Moreover, the impact of these tree species on soil biochemical and microbial properties is poorly known.

The major objective of this study was to assess whether the four commonly used support trees of black pepper (*A. triphysa* (Dennst.) Alston., *E. variegata* L., *G. sepium* (Jacq.) Steud., and *G. pinnata* Roxb.,) had species-specific effects on an array of soil properties that provide a better indication of their rhizosphere health. We hypothesized that these tree species would markedly influence soil organic carbon (SOC) and nutrient levels in the rhizosphere. Specifically, we expected the tree rhizospheres to influence soil nutrient availability and SOC levels, which would significantly alter the microbial biomass, respiration rates and enzyme activities.

2. Materials and methods

2.1. Study site

The study site was located in the Regional Agricultural Research Station (Kerala Agricultural University), Ambalavayal situated in Wayanad district (North latitude 11° 27' and 15° 58' and East longitude 75° 47' and 70° 27'), Kerala State, India. The mean average rainfall in this region is 2322 mm. The mean maximum and minimum temperature for the last five years were 29 °C and 18 °C respectively. This place experiences a high relative humidity, which goes even up to 95% during the Southwest monsoon (June–September) period. Generally the year at the study site is classified into four seasons, viz., cold weather (December–February), hot weather (March–May), Southwest monsoon (June–September) and Northeast monsoon (October–November). The soil of the study site is very deep, well drained, clayey occurring on moderately sloping high hills and is classified as clayey mixed ustic haplohumult (Krishnan et al., 1996).

2.2. Study species

Tree species of uniform age (5 years, height ~10 m) commonly employed as live supports for trailing black pepper viz., *A. triphysa* (Dennst.) Alston., *E. variegata* L., *G. sepium* (Jacq.) Steud. and *G. pinnata* Roxb., were identified for the study. The study area was ~5 ha and included all the tree species under study. A brief description of the steps involved during establishment of the black pepper plantation is as follows. The stem cuttings of tree species (~1 m in length and more than 5 cm in dia.) were collected and stacked in shade and allowed to sprout. The sprouted cuttings were planted at a spacing of 3 × 3 m at a depth of 40–50 cm in May–June. For planting black pepper, pits of size 50 × 50 × 50 cm were made on the northern side of standards, 15 cm away from it. The pits were filled with a mixture of topsoil and FYM (farmyard manure) at 5 kg pit⁻¹. With the onset of southwest monsoon in June–July, 2–3 rooted cuttings of black pepper were planted in the pits at a distance of about 30 cm away from the support. The soil around the cuttings was pressed to form a small mound slopping outward and away from the cuttings to prevent water stagnation around the plants. The growing shoots of the black pepper cuttings were trailed and tied to the support trees. To provide optimum light to the vines and to enable the support trees to grow straight, lopping of the branches of the tree species was done twice a year, once before the onset of monsoon (March–April) and the second after the end of the monsoon (September–October). A non living support made of granite was also included in the study and will henceforth be designated as control. The application of FYM at 5 kg pit⁻¹ was done every year by incorporating the manure around the tree-black pepper rhizosphere at the onset of monsoon.

2.3. Soil sampling

The soils strongly adhering to the roots and within the space explored by the roots was considered as the rhizosphere soil (Garcia et al., 2005). Soil samples were collected from six randomly selected trees (six replications) under each species just before the onset of south west monsoon (June–July) and before the annual incorporation of FYM. In case of control, the soil from six randomly selected granite poles were drawn from 15 cm radius from the pole and 50 cm deep from the top. In the laboratory, the living plant material and coarse roots were removed prior to estimation of moisture content in the samples. A portion of each sample required for estimating the biochemical/ microbial parameters was stored at 4 °C.

2.4. Soil analyses

2.4.1. Soil physico-chemical parameters

The relevant soil physico-chemical parameters were determined in air-dried soils ground to pass a 2 mm sieve. Soil pH was determined in a 1:2.5 soil:water suspension and soil organic C (SOC) by the dichromate oxidation method (Nelson and Sommers, 1996). Mineral N was extracted with 2 M KCl and (1:10 soil:extractant ratio for 1 h) and ammonium and nitrate N were determined by the method described by Mulvaney (1996). Bray P in soils was determined by the method described by Kuo (1996) and exchangeable K by the NH₄OAc method described by Helmke and Sparks (1996). Exchangeable-Ca and -Mg were estimated by the NH₄OAc method described by Suarez (1996) and DTPA extractable micronutrients (Zn, Cu, Mn and Fe) by the method of Lindsay and Norvell (1978). Exchangeable-K, -Ca, -Mg and micronutrients in the extracts were estimated using an atomic absorption spectrophotometer (Varian AA 240FS).

2.4.2. Soil biochemical/microbial parameters

The parameters indicating microbial activity in soils viz., microbial biomass-C (C_{MIC}), -N (N_{MIC}), -P (P_{MIC}), soil respiration and activities of

enzymes in soils were determined in fresh soil samples. Soil respiration was measured as the CO₂ evolved from moist soil, adjusted to 55% water holding capacity, and pre-incubated for seven days at 20 °C in the dark. The CO₂ production was then measured for the next seven days using NaOH traps and titration with HCl (Salamanca et al., 2002). Dissolved organic carbon (DOC) and nitrogen (DON) were determined by the method described by Smolander and Kitunen (2002).

The fumigation–extraction method (Vance et al., 1987) was used to determine soil C_{MIC}, N_{MIC} and P_{MIC} using *k*_{EC} of 0.45 (Joergensen, 1996), *k*_{EN} of 0.54 (Joergensen and Mueller, 1996) and *k*_{EP} of 0.40 (Brookes et al., 1982), respectively. Soil dehydrogenase was assayed using 2, 3, 5-triphenyltetrazolium chloride as the substrate (Casida et al., 1964), urease using urea as the substrate (Kandeler and Gerber, 1988), β-glucosidase using *p*-nitrophenyl-β-D-glucopyranoside as the substrate (Eivazi and Tabatabai, 1988), acid phosphatase using *p*-nitrophenyl phosphate as the substrate (Tabatabai and Bremner, 1969) and arylsulfatase using *p*-nitrophenyl sulfate as the substrate (Tabatabai and Bremner, 1970).

Nitrogen mineralization capacity was determined by extracting 10 g soil with 50 ml of 2 M KCl for 30 min before and after incubation for 10 days at 30 °C. The NH₄-N and total inorganic N were determined by steam distillation (Mulvaney, 1996). The difference between the values obtained before and after incubation indicates N mineralization capacity. Steam distillation for the determination of inorganic N was done using an N analyzer (Kjeltech 2100, Foss).

2.5. Statistics

Analyses for various properties were performed on all soil samples, mean values determined, and the values are expressed on an oven-dry soil basis (24 h at 105 °C). The significance of treatment effects was determined by one-way analysis of variance (ANOVA). Where the *F* values were significant, post hoc comparisons were made using the least significant difference test at the 0.05 probability level. Pearson correlation was used to examine the relationship between two parameters and principal component analysis (PCA) was performed for reflection of any intrinsic pattern in the multidimensional data swarm. PCA often reveals previously unexpected associations among variables and thereby allows interpretation that would not be possible otherwise. Only principal components with Eigen values of more than one (> 1) and that explain >10% of the total variance were retained. A Varimax rotation was performed to enhance the interpretability of the unrelated components (Flury and Riedwyl, 1988). All statistical analyses were performed with STATISTICA 5.1 (Statsoft., 1997).

3. Results and discussion

3.1. Soil pH, SOC and DOC

The data on soil physico-chemical parameters is given in Table 1. In general, soil pH in the rhizosphere of all tree species was acidic (range 4.16–4.62), while the soil in the control had slightly higher pH (5.86). This general trend of soil acidification by trees has been observed under a variety of circumstances (Augusto et al., 2002; Jobbágy and Jackson, 2003; Sinha et al., 2009) and has been attributed to release of H⁺ ions from the respiration of plant roots/soil microorganisms (Hinsinger et al., 2006) or release of acidic exudates in the rhizosphere (Hagen-Thorn et al., 2004).

Contrary to soil pH, the rhizosphere of all tree species showed markedly higher SOC levels than the control soil. In fact, SOC levels were greater by 44.0–77.0% in the tree rhizospheres. Such accumulation of SOC in the tree rhizosphere is of utmost importance because soils in black pepper plantations of the tropics are inherently low in C and nutrient reserves. Among the tree species, greater levels of SOC were registered by the N fixing *G. sepium*, while the lowest level was registered by *A. triphysa*. This confirmed the finding that soils under N fixing trees accumulate C faster than those under other types of trees (Resh et al., 2002).

Similar to SOC, the DOC levels were also markedly higher in the tree rhizosphere compared to the control (Table 2). Among the tree species, DOC levels were highest for *G. pinnata* and *G. sepium*, while the lowest level was registered by *E. variegata*. In the control, the DOC level was lower by 51.4–55.5% compared to the tree rhizospheres. DOC has been proposed as an indicator of carbon available to soil microorganisms (Boyer and Groffman, 1996) and greater levels in the tree rhizosphere suggested release of labile C from roots to soil via sloughed cells and root and mycorrhizal exudates (Marschner, 1995).

3.2. Soil mineral N, DON and total N mineralized

Higher mineral N and DON levels (Tables 1 and 2) also corresponded to the rhizosphere of *G. sepium* and *G. pinnata*. The rhizosphere of *E. variegata* and *A. triphysa* registered lower levels of mineral N and DON, although in both cases they were still higher than the control. In general, in the control, mineral N levels decreased by 54.0–67.0% and DON levels by 36.0–50.0% in comparison to the tree rhizospheres. The inorganic N was predominantly ammoniacal (Table 2) and the tree species registered greater total inorganic N (range 74.5–112.3 mg N kg⁻¹ per 10 days) compared to the control (46.8 mg N kg⁻¹ per 10 days). The results indicated that roots and rhizosphere processes may increase nutrient availability in soils, as suggested by reports of greater N and P

Table 1
Soil pH, organic C and nutrient levels in the rhizosphere of tree species used in black pepper plantations of Kerala, India.

| | Ailanthus ^a | Gliricidia ^b | Garuga ^c | Erythrina ^d | Control |
|--|------------------------|-------------------------|---------------------|------------------------|---------|
| pH (1:2.5 H ₂ O) | 4.52bc | 4.16de | 4.21 cd | 4.62b | 5.86a |
| Organic C (g kg ⁻¹) | 21.6 cd | 26.5a | 24.8b | 22.4c | 15.0e |
| Mineral N (mg kg ⁻¹) | 56 cd | 78a | 74ab | 63c | 26e |
| Bray P (mg kg ⁻¹) | 5.6c | 6.8b | 8.4a | 5.2d | 2.2e |
| Exchangeable K (mg kg ⁻¹) | 322.0a | 233.0 cd | 236.0de | 297.0b | 240.0c |
| Exchangeable Ca (mg kg ⁻¹) | 360.0e | 540.0bc | 573.0b | 456.0d | 643.0a |
| Exchangeable Mg (mg kg ⁻¹) | 96.0e | 118.0c | 113.0 cd | 131.0b | 165.0d |
| Available Zn (mg kg ⁻¹) | 1.8c | 2.7a | 2.6ab | 1.8c | 1.3d |
| Available Cu (mg kg ⁻¹) | 6.8e | 16.0b | 18.3a | 14.2c | 13.6 cd |
| Available Fe (mg kg ⁻¹) | 88ab | 80 cd | 82c | 92a | 76de |
| Available Mn (mg kg ⁻¹) | 4.8de | 10.6a | 8.9b | 8.4bc | 5.4d |

Means followed by the same letter in a row are not statistically significant at *P*<0.05.

^a *Ailanthus triphysa* (Dennst.) Alston, DC.

^b *Gliricidia sepium* (Jacq.) Steud.

^c *Garuga pinnata* Roxb.

^d *Erythrina variegata* L.

Table 2

Biochemical properties of soils in the rhizosphere of tree species used in black pepper plantations of Kerala, India.

| | Ailanthus | Gliricidia | Garuga | Erythrina | Control |
|--|-----------|------------|---------|-----------|---------|
| Dissolved organic C (mg kg ⁻¹) | 398.2bc | 419.6a | 402.5b | 384.2c | 186.8d |
| Dissolved organic N (mg kg ⁻¹) | 44.6ab | 45.8a | 43.2ab | 35.6c | 22.8d |
| Total inorganic N mineralized (mg N kg ⁻¹ per 10 days) | 78.6c | 112.3a | 103.4ab | 74.5 cd | 46.8e |
| Dehydrogenase (nmol TPF g ⁻¹ soil h ⁻¹) | 234c | 244ab | 249a | 221d | 126e |
| Urease (μmol NH ₃ -N g ⁻¹ h ⁻¹) | 7.3de | 8.2a | 7.9ab | 7.4 cd | 7.6bc |
| Acid phosphatase (μmol <i>p</i> -nitrophenol g ⁻¹ h ⁻¹) | 12.9b | 12.7bc | 13.9a | 12.7bc | 7.9d |
| Arylsulfatase (μmol <i>p</i> -nitrophenol g ⁻¹ h ⁻¹) | 0.56ab | 0.52c | 0.58a | 0.45de | 0.48d |
| β-glucosidase (μmol <i>p</i> -nitrophenol g ⁻¹ h ⁻¹) | 7.4 cd | 7.8bc | 9.4a | 8.3b | 5.5e |

Means followed by the same letter in a row are not statistically significant at $P < 0.05$.

availability in the rhizosphere relative to the bulk soil (Priha et al., 1999; Phillips and Fahey, 2006; Phillips and Fahey, 2008). Our results indicated that the rhizosphere of tree species created conditions that promoted an increase in the easily mineralizable inorganic-N pool, which was predominantly ammoniacal. Root-induced stimulation of N mineralization has been reported for seedlings (Priha et al., 1999), and mature trees in forest soils (Phillips and Fahey, 2006; Phillips and Fahey, 2008). The accumulation of mineral N, DON and N mineralized in the rhizosphere was, however, related to tree species. Greater levels were observed in the *G. sepium* rhizosphere possibly because it is a leguminous tree capable of fixing N. Higher rates of N mineralization in the rhizosphere of N fixing trees (Sinha et al., 2009) and varied effects of tropical trees on N accumulation in soils (Ewel, 2006) have been reported.

3.3. Bray P and exchangeable cations (K, Ca and Mg)

Bray P levels were significantly higher in the tree rhizospheres especially the *G. pinnata* rhizosphere (Table 1) suggesting a decrease by 58.0–74.0% in the control. Root induced stimulation of P availability (Phillips and Fahey, 2006) could be one of the reasons for enhanced levels in the rhizosphere of our tree species. Presumably, roots release phosphatase enzymes and low molecular weight organic acids to access P (Hinsinger, 1998). Besides, rhizosphere microbes including mycorrhizal fungi release phosphatase enzymes, which may further contribute to enhanced P availability in the rhizosphere (Marschner, 1995). In our study, the activity of acid phosphatase was markedly higher in the tree rhizosphere relative to control, suggesting a positive rhizosphere effect on phosphatase activity and therefore P availability. Radersma and Grierson (2004) observed two to five times greater acid phosphatase activity in the rhizosphere of several agroforestry trees compared with the bulk soil and concluded that organic anion exudation and acid phosphatase activity of tree roots may increase mobilization of P in the rhizosphere.

In case of exchangeable cations (Table 1), exchangeable-K level was greater in the *A. triphysa* rhizosphere, -Ca in the *E. variegata* rhizosphere and -Mg in the *G. sepium* rhizosphere. The levels of these exchangeable cations in the tree rhizosphere were, however, not always higher than the control. For instance, exchangeable K levels in *G. sepium* and *G. pinnata* rhizospheres were at par with the level in the control. Also, the levels of exchangeable Ca and Mg were markedly lower in the *A. triphysa* rhizosphere compared to the control soil. The tree rhizosphere, therefore, had a negative influence on the levels of exchangeable -Ca and -Mg, possibly due to leaching caused by acid deposition or exudation of low-molecular-weight organic acids by the roots (Schroth et al., 2007) and uptake and sequestering in woody biomass (Huntington et al., 2000).

3.4. Available micronutrients (Zn, Cu, Fe and Mn)

Higher available Zn, Mn and Cu levels corresponded to the rhizosphere of *G. pinnata* and *G. sepium* and higher Fe levels corresponded to the rhizosphere of *E. variegata* and *A. triphysa* (Table 1). This is apparently due

to increased mobilization of micronutrients due to rhizosphere acidification and due to complexities with organic acids in root exudates (Marschner, 1995; Bolan and Duraisamy, 2003). Plant-induced mobilization of trace element speciation and bioavailability in the rhizosphere are the result of sharp biogeochemical gradients in elemental concentrations, pH, $p\text{CO}_2$, $p\text{O}_2$, redox potential and organic ligand concentrations and microbial biomass (Martínez-Alcalá et al., 2009).

In general, our study indicated that the levels of soil nutrients showed marked differences between tree rhizospheres. Earlier studies have also found tree species to affect soil chemical properties such as pH, organic C and rates of N mineralization (Augusto et al., 2002; Russell et al., 2007; Phillips and Fahey, 2008). These species-specific effects are caused by inter-specific differences in organic acid exudation, nutrient uptake, litter quality or quantity, decomposition rates or nutrient output and pumping of nutrients from deep soil (Jobbágy and Jackson, 2004; Hagen-Thorn et al., 2004; Russell et al., 2007). In stands of different tree species established in soils of similar macro-environmental conditions (slope, aspect, elevation, and bedrock), Ayres et al. (2009) found differences in belowground properties among stands of tree species to be the result of differences in tree species traits. Since our study site were also identical in macro-environmental conditions, it seems likely that the tree species were primarily driving the differences that we detected in the soil. Hence, the difference could in part also be attributed to differences in tree species growth rates (Ayres et al., 2004). The rhizosphere of *G. sepium*, a fast growing pioneer species (Sumberg, 2004) probably exuded more C and therefore, showed greater accumulation of SOC and organic substrates followed by *G. pinnata*, *E. variegata* and *A. triphysa* in that order.

3.5. Microbial biomass-C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC})

C_{MIC} and N_{MIC} varied appreciably among the tree species, and the levels were highest for *G. sepium* and *G. pinnata* and lowest for *E. variegata* (Table 3). In contrast, P_{MIC} levels showed little variation among the tree species. However, the C_{MIC} , N_{MIC} and P_{MIC} levels under the tree species were greater than the respective levels in the control. In the control, C_{MIC} levels decreased by 51.0–57.0%, N_{MIC} by 61.0–66.0% and P_{MIC} by 47.0–51.0% compared to the respective levels in the tree rhizospheres. Because soil microbial biomass is considered a temporally integrated measure of labile C (Zak et al., 1993), differences in rhizosphere and control can be attributed to differences in the amount of root-derived C (Phillips and Fahey, 2008), stimulatory effects of nutrients on the rhizosphere microbes (Bradley and Fyles, 1996) and increased C flux in the rhizosphere (Uselman et al., 2000). This is well reflected by the existence of a strong correlation ($P < 0.05$; $n = 30$) between C_{MIC} and SOC ($r = 0.82$), DOC ($r = 0.85$) and DON ($r = 0.82$). The tree species, however, varied markedly in their effects on microbial biomass levels possibly due to variation in the levels of exudates, secretions, sloughed cells and other debris from fine roots to soil or due to differences in organic matter composition (McMillan et al., 2007). Alvarez et al. (2009) reported that factors other than organic matter quantity (i.e. organic matter quality, nutrient availability, moisture

Table 3

Microbial biomass-C (C_{MIC}), -N (N_{MIC}), -P (P_{MIC}), soil respiration and ratios of various microbial indices in the rhizosphere of tree species used in black pepper plantations of Kerala, India.

| | Ailanthus ^a | Gliricidia ^b | Garuga ^c | Erythrina ^d | Control |
|---|------------------------|-------------------------|---------------------|------------------------|---------|
| C_{MIC} ($\mu\text{g g}^{-1}$) | 423c | 474a | 454ab | 415 cd | 205e |
| N_{MIC} ($\mu\text{g g}^{-1}$) | 28b | 32a | 29ab | 29ab | 11c |
| P_{MIC} ($\mu\text{g g}^{-1}$) | 14.7a | 13.6a | 14.2a | 14.0a | 7.2b |
| Soil respiration ($\mu\text{g CO}_2\text{-C g}^{-1}\text{ day}^{-1}$) | 25.0a | 24.0ab | 24.0ab | 22.0bc | 17.0d |
| C_{MIC} :organic C (%) | 1.96a | 1.79 cd | 1.83bc | 1.85b | 1.37e |
| C_{MIC} : N_{MIC} | 15.1b | 14.8b | 14.6b | 14.3b | 18.6a |
| C_{MIC} : P_{MIC} | 28.8a | 34.8a | 32.0a | 29.6a | 28.4a |
| $q\text{CO}_2$ ($\text{mg CO}_2\text{-C (g biomass C)}^{-1}\text{ day}^{-1}$) | 59b | 51 cd | 53c | 53c | 83a |

Means followed by the same letter in a row are not statistically significant at $P < 0.05$.

^a *Ailanthus triphysa* (Dennst.) Alston. DC.

^b *Gliricidia sepium* (Jacq.) Steud.

^c *Garuga pinnata* Roxb.

^d *Erythrina variegata* L.

content, microbial diversity, etc.) were responsible for variations in microbial biomass levels among tree species. Perhaps the root exudates of *G. sepium* and to a certain extent *G. pinnata* stimulated rhizosphere microbial activity, both furnishing organic substrates and creating a more favourable microhabitat.

From the results it is evident that decreased levels of biologically available substrates and organic matter lead to simultaneous decrease in microbial activity in the control. This was well reflected by the C_{MIC} :SOC ratios, which ranged from 1.37–1.96% (Table 3). The C_{MIC} :OC ratios were greatest for tree species (range 1.96–1.79%) and least for the control (1.37%). Lower C_{MIC} :SOC ratio in the control suggested lower availability and/or degradability of organic substrates and, therefore, decreased microbial development and C turnover. In contrast, C_{MIC} : N_{MIC} ratio was greatest for control (18.6) and least for the tree species (14.3–15.1) possibly due to low N availability in combination with relatively higher organic matter availability as observed by Salamanca et al (2002) in some secondary tropical forest sites of Philippines. The C_{MIC} : P_{MIC} ratios were greatest for *G. sepium* (34.8) and *G. pinnata* (32.0) and almost identical for *A. triphysa*, *E. variegata* and control (28.8, 29.6 and 28.4 respectively).

3.6. Soil respiration and metabolic quotient ($q\text{CO}_2$)

Unlike microbial biomass, soil respiration rates did not vary significantly among the tree species and lowest soil respiration was registered in the control (Table 3). Respiration rates decreased by 23.0–32.0% in the control compared to the tree rhizospheres. Soil respiration is a useful parameter for measuring the biological activity of the soil (Grayston et al., 2001) and enhanced rates in tree rhizosphere can be attributed to greater levels of SOC, which has been found to account for 75.0 and 81.0% of the variations in CO_2 production in the non-preincubated and pre-incubated soils respectively (Wang et al., 2003). Differences in soil respiration among the soils were also indicative of variable amounts of labile SOC accumulated among different tree species (Masto et al., 2006). In our study, the respiration rates were positively correlated to SOC ($r = 0.80$, $p < 0.05$, $n = 30$), thus confirming the close relationship between these two parameters (Dube et al., 2009). The respiration rates were also positively correlated to C_{MIC} ($r = 0.78$, $P < 0.05$, $n = 30$) indicating that in tree rhizospheres, respiration by root systems and their microbial biomass components would also contribute significantly to soil respiration (Keltling et al., 1998).

Unlike soil respiration, metabolic quotient ($q\text{CO}_2$) was markedly higher in the control ($83 \text{ mg CO}_2\text{-C (g biomass C)}^{-1}\text{ day}^{-1}$) and least in the rhizosphere of tree species ($51\text{--}59 \text{ mg CO}_2\text{-C (g biomass C)}^{-1}\text{ day}^{-1}$; Table 3). The $q\text{CO}_2$, which has been used as a bioindicator for environmental stress (Anderson and Domsch, 1993), reflects the efficiency of the use of SOC by microorganisms (Knoepp et al., 2000). The decrease of $q\text{CO}_2$ under the tree species especially *G. sepium* suggests less adverse environmental conditions and higher use

efficiency of the organic resources than in the control. Contrarily, higher $q\text{CO}_2$ in the control indicated that the conversion of total carbon into microbial carbon is less efficient as reported by Frazão et al. (2010) in soils of varying land use. Presumably, in the control soil, shifts occurred in the ecological efficiency of the microbial community due to decrease in the labile substrate pool like DOC, DON etc. The more efficiently the microorganisms utilize the substrate, the greater the fraction of substrate C that is incorporated into microbial biomass and the less C per unit biomass that is lost through respiration, which resulted in a low $q\text{CO}_2$ (Ilstedt et al., 2003). Among the tree species, higher $q\text{CO}_2$ level in *A. triphysa* probably indicated its relatively poor rhizosphere health.

3.7. Soil enzyme activities

There is currently great interest in the use of dehydrogenase and extracellular hydrolytic enzymes as the most appropriate indicators of microbial function and soil fertility (Cadwell, 2005) and the results revealed that the enzymes were activated to varying degrees in the rhizosphere of tree species. Dehydrogenase, which indicates the overall metabolic activity (Quilchano and Marañón, 2002) was highest for *G. sepium*, and *G. pinnata* (244.0 and $249.0 \text{ nmol TPF g}^{-1}\text{ soil h}^{-1}$ respectively) and the activity under the control ($126 \text{ nmol TPF g}^{-1}\text{ soil h}^{-1}$) indicated a 43.0–49.0% decrease in comparison to the tree rhizospheres (Table 2). This confirmed that the microbial biomass is biologically more active in the tree rhizosphere principally because of higher amounts of SOC/substrates entering the soil system. This was evident from the positive correlation ($p < 0.05$, $n = 30$) between dehydrogenase activity and relevant parameters like SOC ($r = 0.70$), soil respiration ($r = 0.69$) and C_{MIC} ($r = 0.71$).

The activities of hydrolases involved in C (β -glucosidase), N (urease), P (acid phosphatase) and S (arylsulfatase) cycles in soils were also markedly higher in the tree rhizosphere compared to the respective levels in the control. In the control, the per cent decrease in activity of acid phosphatase ranged between 37.6 and 43.0 and β -glucosidase between 24.8 and 41.3. Urease activity was greatest in *G. sepium* and *G. pinnata* (8.2 and $7.9 \mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$ respectively), but was almost identical under *A. triphysa*, *E. variegata* and control. However, not all the tree species affected the enzyme activities to the same degree. For instance, the rhizosphere of *G. sepium* registered the highest urease activity ($8.2 \mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$) and *G. pinnata* rhizosphere the highest β -glucosidase and acid phosphatase activity (9.4 and $13.9 \mu\text{mol p-nitrophenol g}^{-1}\text{ h}^{-1}$ respectively). Arylsulfatase activity was greatest in the *G. pinnata* and *A. triphysa* rhizosphere (0.58 and $0.56 \mu\text{mol p-nitrophenol g}^{-1}\text{ h}^{-1}$ respectively) and was almost identical in the *E. variegata* rhizosphere and control. Such differences in biochemical parameters between rhizosphere soil and non-rhizosphere

soil have been previously described by Caravaca et al. (2003) in an abandoned stipa-grass cropping system.

Our results indicated that the rhizosphere induces the synthesis of these enzymes as a consequence of enhanced substrate and microbial biomass levels (Caravaca et al., 2005). This was clearly evident from the positive correlations ($p < 0.05$, $n = 30$) we obtained between SOC content and activities of β -glucosidase ($r = 0.80$) and acid phosphatase ($r = 0.78$). In agreement with our results many studies have formerly described positive correlations between enzyme activities and SOC (Rodríguez-Loínaz et al., 2008; Enowashu et al., 2009). Most of the variation in enzyme activities among the tree species can, therefore, be explained by C_{MIC} and SOC, which appears logical because enzymes are synthesized by soil microorganisms in response to the presence of suitable substrate.

3.8. Inter-relationships between various soil parameters

This was studied using PCA (Table 4) wherein, the Factor I (PC1) explaining 67.7% of the total variance was defined mainly by SOC, mineral N, N mineralized, DOC, DON, dehydrogenase, acid phosphatase, β -glucosidase, C_{MIC} , N_{MIC} , P_{MIC} and soil respiration. This reflected the size and activity of the microbial biomass and possibly reflected the strong relationship between the availability of labile and easily mineralisable organic matter and the activity of microbial populations. The high loadings of mineral N and Bray P in the first factor indicated the logical dependence of microbial biomass on soil nutrients. The inclusion of soil respiration, P_{MIC} , phosphomonoesterase and arylsulphatase in this factor indicated the strong relationship between microbial activity and N/S cycles in soils. Also the higher loadings of β -glucosidase suggested that in the tree rhizosphere the potential to mineralise organic matter, and so the activity of the C cycle, is enhanced. The negative loading of qCO_2 in this factor suggested a decrease in substrate use efficiency in soils with low organic matter especially the control. Also, the negative loading of pH in this factor indicated that release of acidic exudates and root respiration reduced the soil pH in the rhizosphere. Factor II (PC2) explaining 20.5% of the total variance was defined mainly by urease and somewhat lower positive loadings of SOC, mineral N and N mineralized, reflecting the

Table 4

Principal component loadings after Varimax rotation. The soil parameters are grouped according to the maximum fittings to principal components (Correlation coefficients > 0.50 ; $n = 30$).

| Measurements | Principal components ^a | |
|---|-----------------------------------|--------------|
| | PC1 | PC2 |
| Eigen values | 13.55 | 1.45 |
| Explained variance (%) | 67.7 | 20.5 |
| Rotated loading on two retained components ^b | | |
| pH | -0.87 | -0.52 |
| Organic C | 0.78 | 0.56 |
| Mineral N | 0.79 | 0.54 |
| N mineralized | 0.66 | 0.72 |
| Bray P | 0.76 | 0.42 |
| Dissolved organic C | 0.86 | 0.25 |
| Dissolved organic N | 0.83 | 0.35 |
| Dehydrogenase | 0.83 | 0.27 |
| Urease | -0.05 | 0.87 |
| Acid phosphatase | 0.81 | 0.04 |
| Aryl sulphatase | 0.43 | 0.35 |
| β -glucosidase | 0.78 | 0.33 |
| C_{MIC} | 0.84 | 0.31 |
| N_{MIC} | 0.81 | 0.23 |
| P_{MIC} | 0.73 | 0.08 |
| Soil respiration | 0.83 | 0.15 |
| qCO_2 | -0.84 | -0.31 |

^a Only principal components with eigen values > 1 and those explaining $> 10\%$ of the total variance were retained.

^b Correlations with absolute values higher than 0.50 are in bold.

strong relationship between urease activity, N dynamics and organic matter mineralization in soils.

4. Conclusions

Our study demonstrated that the rhizosphere of tree species used as supports in black pepper plantations differed in their effects on soil nutrient availability, enzyme and microbial activities. Variations in microbial and enzyme activities were associated with differences in the levels of nutrients and SOC. The study further indicated that the rhizosphere soils under tree species are more active and root exudates have a profound influence on the soil properties. Consequently, each tree species may modify the conditions in the rhizosphere in order to maximize nutrient acquisition from organic matter. The rhizospheres of *G. sepium* and *G. pinnata* possessed greater nutrient, SOC and substrate levels and hence supported higher microbial biomass and enzyme activity. The results imply that in the plains of the humid tropics, *G. sepium* and to some extent *G. pinnata* could be preferred as live support trees for restoration of degraded black pepper plantations, development of sustainable black pepper-tree support systems and enhancement of soil quality.

Acknowledgements

We thank the Director, IISR, Calicut, Kerala, India for the facilities provided to carry out this work. We are grateful to the staff of RARS (Kerala Agricultural University), Ambalavayal, Kerala, India for help with soil sampling. We are also grateful to the Study Circle of IISR for the suggestions offered to improve this MS No 439.

References

- Alvarez, E., Torrado, V.M., Fernández Marcos, M.L., Diaz-Raviña, M., 2009. Microbial biomass and activity in a forest soil under different tree species. *EJEAFChe*, 8, 878–887.
- Anderson, T.-H., Domsch, K.H., 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* 25, 393–395.
- Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. *Ann. For. Sci.* 59, 233–253.
- Ayres, E., Heath, J., Possell, M., Black, H.I.J., Kerstiens, G., 2004. Tree physiological responses to above-ground herbivory directly modify below-ground processes of soil carbon and nitrogen cycling. *Ecol. Lett.* 7, 469–479.
- Ayres, E., Steltzer, H., Berg, S., Wallenstein, M.D., Simmons, B.L., Wall, D.H., 2009. Tree species traits influence soil physical, chemical, and biological properties in high elevation forests. *PLoS ONE* 4, e5964.
- Bolan, N.S., Duraisamy, V.P., 2003. Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: a review involving specific case studied. *Aust. J. Soil Res.* 41, 533–555.
- Boyer, J.N., Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. *Soil Biol. Biochem.* 28, 783–790.
- Bradley, R.L., Fyles, J.W., 1995. Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil nutrients. *Soil Biol. Biochem.* 27, 1565–1571.
- Bradley, R.L., Fyles, J.W., 1996. Interactions between tree seedling roots and humus forms in the control of soil C and N cycling. *Biol. Fertil. Soils* 23, 70–79.
- Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14 (3), 19–329.
- Cadwell, B.A., 2005. Enzyme activities as component of soil biodiversity: a review. *Pedobiol.* 49, 637–644.
- Caravaca, F., Figueroa, D., Barea, J.M., Azcón-Aguilar, C., Palenzuela, J., Roldán, A., 2003. The role of relictic vegetation in maintaining physical, chemical and biological properties in an abandoned *Stipa*-grass cropping system. *Arid Land Res. Manag.* 17, 103–111.
- Caravaca, F., Alguacil, M.M., Torres, P., Roldán, A., 2005. Plant type mediates the rhizosphere microbial activities and soil aggregation in a semiarid Mediterranean salt marsh. *Geoderma* 124, 375–382.
- Casida Jr, L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. *Soil Sci.* 98, 371–376.
- Dube, F., Zagal, E., Stolpe, N., Espinosa, M., 2009. The influence of land-use change on the organic carbon distribution and microbial respiration in a volcanic soil of the Chilean Patagonia. *For. Ecol. Manage.* 257, 1695–1704.
- Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. *Soil Biol. Biochem.* 20, 601–606.

- Enowashu, E., Poll, C., Lamersdorf, N., Kandeler, E., 2009. Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. *Appl. Soil Ecol.* 43, 11–21.
- Ewel, J., 2006. Species and rotation frequency influence soil nitrogen in simplified tropical plant communities. *Ecol. Appl.* 16, 490–502.
- Flury, B., Riedwyl, H., 1988. *Multivariate statistics. A practical approach*, Chapman and Hall, London.
- Frazão, L.A., Piccolo, M.C., Feigl, B.J., Cerri, C.C., Cerri, C.E.P., 2010. Inorganic nitrogen, microbial biomass and microbial activity of a sandy Brazilian Cerrado soil under different land uses. *Agric. Ecosyst. Environ.* 135, 161–167.
- García, C., Roldán, A., Hernandez, T., 2005. Ability of different plant species to promote microbiological processes in semiarid soil. *Geoderma* 124, 193–202.
- Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol. Biochem.* 33, 533–551.
- Hagen-Thorn, A., Callesen, I., Armolaitis, K., Nihlgård, B., 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. *For. Ecol. Manage.* 195, 373–384.
- Helmke, P.A., Sparks, D.L., 1996. Lithium, potassium, rubidium, and caesium. In: Sparks, D.L. (Ed.), *Methods of soil analysis, Part 3, Chemical methods*, SSSA Book Series no. 5, Madison, WI, pp. 551–574.
- Hinsinger, P., 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Adv. Agron.* 64, 225–265.
- Hinsinger, P., Plassard, C., Jaillard, B., 2006. Rhizosphere: A new frontier for soil biogeochemistry. *J. Geochem. Explor.* 88, 210–213.
- Huntington, T.G., Hooper, R.P., Johnson, C.E., Aulenbach, B.T., Cappellato, R., Blum, A.E., 2000. Calcium depletion in a southeastern United States forest ecosystem. *Soil Sci. Soc. Am. J.* 64, 1845–1858.
- Ilstedt, U., Giesler, R., Nordgren, A., Malmer, A., 2003. Changes in soil chemical and microbial properties after a wildfire in a tropical rainforest in Sabah, Malaysia. *Soil Biol. Biochem.* 35, 1071–1078.
- Jobbágy, E.G., Jackson, R.B., 2003. Patterns and mechanisms of soil acidification in the conversion of grasslands to forests. *Biogeochem.* 64, 205–229.
- Jobbágy, E.G., Jackson, R.B., 2004. The uplift of soil nutrients by plants: biogeochemical consequences across scales. *Ecol.* 85, 2380–2389.
- Joergensen, R.G., 1996. The fumigation–extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. *Soil Biol. Biochem.* 28, 25–31.
- Joergensen, R.G., Mueller, T., 1996. The fumigation–extraction method to estimate soil microbial biomass: calibration of the k_{EN} value. *Soil Biol. Biochem.* 28, 33–37.
- Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fertil. Soils* 6, 68–72.
- Kelting, D.L., Burger, J.A., Edwards, G.S., 1998. Estimating root respiration, microbial respiration in the rhizosphere, and root-free respiration in forest soils. *Soil Biol. Biochem.* 30, 961–968.
- Knoepp, J.D., Coleman, D.C., Crossley Jr., D.A., Clark, J.S., 2000. Biological indices of soil quality: an ecosystem case study of their use. *For. Ecol. Manage.* 138, 357–368.
- Krishnan, P., Venugopal, K.R., Sehgal, J., 1996. *Soils of Kerala: their kinds, distribution, characterization and interpretations for optimising land use*. NBSS Publication 48, Soils of India Series. National Bureau of Soil Survey and Land Use Planning, Nagpur, India. pp. 60.
- Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), *Methods of soil analysis, Part 3, Chemical methods*, SSSA Book Series no. 5, Madison, WI, pp. 869–919.
- Lindsay, W.L., Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* 42, 421–428.
- Marschner, H., 1995. *Mineral nutrition of higher plants*, 2nd edition. Academic Press, London.
- Martínez-Alcalá, I., Clemente, R., Bernal, M.P., 2009. Metal availability and chemical properties in the rhizosphere of *Lupinus albus* L. growing in a high-metal calcareous soil. *Water, Air, Soil Pollut* 201, 283–293.
- Masto, R.E., Chhonkar, P.K., Singh, D., Patra, A.K., 2006. Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical inceptisol. *Soil Biol. Biochem.* 38, 1577–1582.
- McMillan, R., Quideau, S.A., Mackenzie, M.D., Biryukova, O., 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. *J. Environ. Qual.* 36, 1470–1478.
- Mulvaney, R.L., 1996. Nitrogen-Inorganic forms. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3, Chemical Methods*, SSSA Book Series no. 5, Madison, WI, pp. 1123–1184.
- Nair, P.K.R., 1993. *An Introduction to Agroforestry*. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3, Chemical Methods*, SSSA Book Series no. 5, Madison, WI, pp. 961–1010.
- Phillips, R.P., Fahey, T.J., 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology* 87, 1302–1313.
- Phillips, R.P., Fahey, T.J., 2008. The influence of soil fertility on rhizosphere effects in northern hardwood forest soils. *Soil Sci. Soc. Am. J.* 72, 453–461.
- Priha, O., Grayston, S.J., Pennanen, T., Smolander, A., 1999. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. *FEMS Microbiol. Ecol.* 30, 187–199.
- Quilchano, C., Marañón, T., 2002. Dehydrogenase activity in Mediterranean forest soils. *Biol. Fertil. Soils* 35, 102–107.
- Radersma, S., Grierson, P.F., 2004. Phosphorus mobilization in agroforestry: organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. *Plant Soil* 259, 209–219.
- Resh, S., Binkley, D., Parrotta, J., 2002. Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. *Ecosystems* 5, 217–231.
- Rodríguez-Loiñaz, G., Onaindia, M., Amezcaga, I., Mijangos, I., Garbisu, C., 2008. Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. *Soil Biol. Biochem.* 40, 49–60.
- Russell, A.E., Raich, J.W., Valverde-Barrantes, O.J., Fisher, R.F., 2007. Tree species effects on soil properties in experimental plantations in tropical moist forest. *Soil Sci. Soc. Am. J.* 71, 1389–1397.
- Salam, A.M., Mohanakumar, N., Jayachandran, B.K., Mamman, B.K., Sreekumar, D., Satheesh Babu, K., 1991. Thirty one tree species support black pepper vines. *Agroforest. Today* 3, 16.
- Salamanca, E.F., Raubuch, N., Joergensen, R.G., 2002. Relationships between soil microbial indices in secondary tropical forest soils. *Appl. Soil Ecol.* 21, 211–219.
- Schroth, A.W., Friedland, A.J., Bostick, B.C., 2007. Macronutrient depletion and redistribution in soils under conifer and northern hardwood forests. *Soil Sci. Soc. Am. J.* 71, 457–468.
- Sinha, S., Masto, R.E., Ram, L.C., Selvi, V.A., Srivastava, N.K., Tripathi, R.C., George, J., 2009. Rhizosphere soil microbial index of tree species in a coal mining ecosystem. *Soil Biol. Biochem.* 41, 1824–1832.
- Smolander, A., Kytönen, V., 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biol. Biochem.* 34, 651–660.
- Srinivasan, V., Dinesh, R., Hamza, S., Parthasarathy, V.A., 2007. Nutrient management in black pepper (*Piper nigrum* L.). *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2, No. 062, pp. 1–14.
- Statsoft.1997. *STATISTICA for Windows Release 5.1*, Tulsa, OK, U.S.A.
- Suarez, D.L., 1996. Beryllium, magnesium, calcium, strontium and barium. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3, Chemical Methods*, SSSA Book Series no. 5, Madison, WI, pp. 575–601.
- Sumberg, J.E., 2004. Collection and initial evaluation of *Gliricidia sepium* from Costa Rica. *Agroforest. Syst.* 3, 357–361.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.
- Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. *Soil Sci. Soc. Am. Proc.* 34, 225–229.
- Turpault, M.P., Gobran, G.R., Bonnaud, P., 2007. Temporal variations of rhizosphere and bulk soil chemistry in a Douglas fir stand. *Geoderma* 137, 490–496.
- Uselman, S.M., Qualls, R.G., Thomas, R.B., 2000. Effects of increased atmospheric CO₂, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant Soil* 222, 191–202.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.
- Wang, W.J., Dalal, R.C., Moody, P.W., Smith, C.J., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biol. Biochem.* 35, 273–284.
- Zak, D.R., Pregitzer, K.S., Curtis, P.S., Teeri, J.A., Fogel, R., Randlett, D.L., 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant Soil* 151, 105–117.