MICROBIAL AND CHEMICAL SOIL PROPERTIES OF SECONDARY FOREST SOILS WITH DIFFERENT VEGETATION AND STAND AGE IN LA UNION, PHILIPPINES

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Abstract

We investigated six sites with the aim to assess the effects of tree plantations with different species and different stand age (18 months to 40 years) on biological functions such as soil organic matter accumulation and especially on the activity, biomass and community structure of soil microorganisms. Soil pH, clay content and cation exchange capacity, have neither significant effects on microbial biomass and activity indices. In contrast, soil organic matter and microbial properties were highly interrelated with correlation coefficients between r = 0.51 (ergosterol and total N) and r = 0.90 (biomass N and soil C). Although all soil organic matter and microbial properties are within the range described in the literature, our six forest sites reveals several interesting and important differences: Average ratios of biomass C-to-soil C were 2.8%, biomass N-to-total N 2.0%, biomass C-to-N 14.1, ergosterol-to-biomass C 0.19%, ATP-to-biomass C 4.3 µmol g^{-1}. AEC and metabolic quotient qCO_2 reached average levels of 0.71 and 36 (mg CO_2-C d^{-1} g^{-1} biomass), respectively. The maximum biomass N-to-total N ratio was observed at the Calliandra site in combination with maximum AEC, qCO_2, ergosterol-to-biomass C ratio, and a minimum biomass C-to-N ratio. The soil C-to-N ratio showed a significant positive correlation with the biomass N-to-total N ratio and a negative with the ATP-to-biomass C ratio. However, the strongest relationship was found between the ratios of biomass N-to-total N and the ergosterol-to-biomass C, followed by the that of biomass C-to-soil C and biomass C-to-N. Also a significant, but negative correlation revealed the biomass C-to-N ratio and the qCO_2. The AEC was positively correlated with the qCO_2 and the biomass N-to-total N ratio with similar levels of significance.

Key words: Biomass N, Biomass C, Ergosterol, ATP, AEC, Respiration

INTRODUCTION

Sustainable productivity of forestland is maintained by microbially mediated soil processes, thus, controlling soil fertility and ecosystem functioning (Smith and Paul, 1990). Soil microorganisms are the driving force for the nutrient supply to plants in agricultural, but also in forestland (Singh *et al.,* 1989). On this premise, it is surprising to note the scarcity of studies on soil biological problems in tropical soils considering the large variety of soils in this region. Since temperature controls many processes in soil, the higher temperature in tropical regions lead to faster turnover rates of microbial biomass or soil organic matter in comparison to temperate Northern Europe, shortening the time for ecosystems to respond to changes in management practices (Grisi *et al*., 1998). Several studies exist for the effects of tree species, stand age and soil type on soil microbial biomass and its activity in Australian boreal forest systems (Bauhus *et al*., 1993, 1998) and in Indian dry tropical forest systems (Singh *et al.,* 1989; Srivastava and Lal, 1994; Patra *et al.,* 1995).

No information exists on the biological properties of secondary forest soils developed from old volcanic deposits under wet monsoonal conditions used for a longer as arable or grassland.

We investigated six sites with the aim to assess the effects of tree plantations with different species and different stand age (18 months to 40 years) on biological functions such as soil organic matter accumulation and especially on the activity, biomass and community structure of soil microorganisms. Microbial activity was measured as basal respiration, i.e. CO_2 production in laboratory under optimum conditions (Anderson and Domsch, 1989), microbial biomass C and N by fumigation extraction (Brookes *et al.,* 1985; Vance *et al.,* 1987) and the community structure by measuring ergosterol, a fungi-specific component of the cell membranes (Montgomery *et al.,* 2000). A second aim was to assess the effects of different species and different stand age in relation with the small-scale variability of soil properties such as clay content, cation exchange capacity, and soil pH.

The sites investigated were located in the 960 ha valley representing University Forest of the Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines. The soils have been developed from the same parent material and similar climatic conditions. The results may improve the scientific basis of forest management decisions on the type of species for planting in the area and, thus, they may enhance the sustainability of land use.

MATERIALS AND METHODS

The study site

The study sites were located in a 960 ha valley representing the University Forest of the Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines 16^{o} 6' N latitude and 120^{o} 2' E longitude. The average annual temperature is 28 ^{o}C and total rainfall in 1998 was 2290 mm and in 1999 was 4070 mm. The area has two distinct seasons, wet from June to October and dry from November to April. The percentage of rainfall during the wet season is at least 75 per cent of the total precipitation of the year. The amount of clouds coincides with the average precipitation, the clearest months are from January to April and the cloudiest months are from July to September. The annual percentage humidity is not very high (<80 per cent) owing to the influence and duration of the dry season

The soils of the six sampling sites have been developed from the same parent material but they are covered presently with different vegetation at different age. Based on the 1950 soil survey in the province (Alicante *et al*, 1950), the area was described as consisting largely of elevated coastal tract made in part of raised coral and in part of alluvium overlying older sediments. The soil consists of 51.04% clay, 17.51% silt and 31.45% sand. The first 25 cm (0-25 cm) is reddish brown, fine granular slightly compact clay. Consistency varies from sticky, plastic, friable to hard with decreasing moisture content. Roots penetrate easily and are abundant in this layer. Limited amount of well-decomposed organic matter boundary between this and the layer below is wavy and clear. At the 25 to 90 cm, the soil is reddish brown, fine to coarse granular clay heavier in texture than the above layer, intimately mixed with yellowish gray to brownish gray partially weathered corralling limestone, Very few toots go beyond 30 cm deep. The boundary is granular. The soil at 90 to 129 cm is bedrock to coralline limestone.

Site 1 is north facing and predominantly covered with a 20 years old large-leaf mahogany (*Switenia macrophylla*) forest. Site 2 is west facing site and was planted with silver oak (*Grevillea robusta*) trees 1.5 years ago. It was formerly grassland dominated by *Imperata cylindrica*. Site 3 is a pure plantation of 9 years old calliandra (*Calliandra calothyrsus*). The site was formerly a paddy rice field. Site 4 is south facing was planted with kakawate (*Gliricidia sepium*) trees along the contour 15 years ago. The leaves and branches are trimmed down every year and trimmings are placed at the base of the trees. Site 5 is south facing and predominantly covered with roughly 40 years old teak (*Tectona grandis*) trees. Site 6 is south facing and a pure

plantation of 5 years old yemane (*Gmelina arborea*) trees. The upper 100 cm of the soil were scrapped several years prior to planting.

Soil sampling and analysis

At each of the 6 sites, samples were taken from 4 profiles, building a square of 10 m in March 2000 at a depth of 0-10 cm after removal of the litter layer. Bulk samples were sieved (< 2 mm) and transferred within one week to Witzenhausen, Germany, in polyethylene bags and a suitable container. Soil pH was measured in water using a soil-to-water ratio of 1-to-2.5. Clay content was determined by a pipet method after pre-treatment with H_2O_2, Na-citrate and $Na_2S_2O_4$ to remove organic matter and iron oxides (Gee and Bauder, 1986). Cation exchange capacity was measured according to Mehlich in buffered triethanolamine–$BaCl_2$ solution (0.1 M, pH 8.5) (Schlichting *et al.,* 1995). Subsamples of dried soil material were homogenised in a ball mill. Then, soil C and total N were determined using gas chromatography after combustion at 1200 ºC using a Carlo Erba ANA 1400 Analyser.

Microbial soil properties

For the measurement of CO_2 production, 100 g (oven-dry basis) of a moist soil sample was weighed into 1-l stoppered Pyrex jar, adjusted to 55% of its maximum water-holding capacity and pre-incubated for 3 days 20 ºC in dark. Then, CO_2 production was finally measured for another 3 days. The CO_2 evolved was absorbed in 20 ml 0.05 M NaOH solution and determined by titration of the excess NaOH with 0.05 M HCl. The metabolic quotient qCO_2 was calculated as follows: (µg CO_2-C evolved in 3 days g^{-1} soil) / (µg biomass C g^{-1} soil) / 7 days x 1000 = mg CO_2-C d^{-1} g^{-1} biomass C.

Microbial biomass C and biomass N were estimated by fumigation-extraction (Brookes *et al.,* 1985; Vance *et al.,* 1987). Two portions equivalent to 25 g oven-dry soil were taken from the soil sample. One portion was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 100 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (Schleicher & Schuell 595 ½). The non-fumigated portion was extracted similarly at the time fumigation commenced. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatec 100 automatic analyser. Microbial biomass C was calculated as follows: Microbial biomass C = E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu *et al.,* 1990; Joergensen, 1996). Total N in the extracts was measured as NO_2* by chemoluminеszenz detection after combustion at 830 °C using a Dimatec 100 automatic analyser. Microbial biomass N was calculated as follows: Microbial biomass N = E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes *et al.,* 1985; Joergensen and Mueller, 1996).

Ergosterol was measured in moist soil of 2 g dry weight. This sample was extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1} (Djajakirana *et al.,* 1996). Quantitative determination of ergosterol was performed by reversed-phase HPLC analysis at 26 °C using a column of 125 x 4 mm Sphereclone 5µ ODS II with a Phenomenex guard column (4 x 3 mm). The chromatography was performed isocratically with 100% and a resolution of detection of 282 nm (Dionex UVD 170 S).

Measurements of adenine nucleotides and calculations of the adenylate energy charge (AEC) were made according to the procedure of Bai et al. (1989) as described by Dyckmans and Raubuch (1997). Dimethylsulfoxid (DMSO), Na_3PO_4-buffer (10 mM) and NRB (Nucleotide Releasing Reagent for microbial ATP, Lumac, the Netherlands) were used as extractant. After

derivatisation with chloracetaldehyde, the adenine nucleotides were determined by HPLC. The HPLC system consisted of a Gynkotek 480 pump, a Gynkotek GINA 50 automatic sample injector, a Dionex RF 2000 fluorescence detector. The separation was carried out on a 250 x 4.6 mm column (5 µm ODS Hypersil) with guard column (10 x 4.6 mm). The chromatography was performed isocratically with 50 mM ammonium acetate buffer containing 1 mM EDTA and 0.4 mM TBAHS mixed with methanol (89.5/10.5 v/v) as mobile phase. Column temperature was set to 26 °C using a Dionex STH 585 column oven. Fluorometric emission was measured at a wavelength of 410 nm with 280 nm as the excitation wavelength.

Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105 °C). The significance of treatment effects on cumulative CO_2 production and N mineralisation was tested by one-way analysis of variance using the Tukey/Kramer HSD-test (honestly significant difference) using the StatView 5.0 program (SAS Inc.).

RESULTS

Soil pH of the six different sites varied in a slightly acidic range between 5.9 and 6.5. The cation exchange capacity ranged from 379 to 412 $\mu mol_C\ g^{-1}$ soil, the clay content from 28 to 43%. However, these two properties did not reveal any significant difference between the sites due to a considerable spatial variability. It should be noted that the variation of physical, chemical and biological properties is mainly caused by spatial variability and not by analytical error.

In contrast to the clay content and the cation exchange capacity, reflecting both the parent material of the soils, significant differences between some sites occurred for those properties reflecting biological functions such as the soil organic matter accumulation or as biomass and activity rates of soil microorganisms. Soil C ranged from 7.4 to 18.4 mg g^{-1} soil, total N from 0.79 to 1.65 mg g^{-1} soil, and the soil C-to-N ratio from 9.1 to 11. The CO_2 production rate varied between 7.6 and 23.3 µg g^{-1} soil d^{-1} and microbial biomass C between 208 and 487 µg g^{-1} soil. Maximum contents of soil C and total N were observed at the Mahogany site, followed by the Grevillea and Calliandra sites. In contrast to this, maximum microbial biomass or activity indices were found at the Grevillea site (biomass C, ergosterol, AMP, ADP, and ATP) or at the Calliandra site (biomass N and CO_2 production). Minimum values of soil organic matter properties were always found at the Yemane site, except the soil C-to-N ratio that was lowest at the Gliricidia site. In most cases, soil pH, clay content and cation exchange capacity, have neither significant effect on microbial biomass and activity indices (Table 4). In contrast, soil organic matter and microbial properties were highly interrelated with correlation coefficients between r = 0.51 (ergosterol and total N) and r = 0.90 (biomass N and soil C).

Average ratios of biomass C-to-soil C were 2.8%, biomass N-to-total N 2.0%, biomass C-to-N 14.1, ergosterol-to-biomass C 0.19%, ATP-to-biomass C 4.3 µmol g^{-1}. AEC and metabolic quotient qCO_2 reached average levels of 0.71 and 36 (mg CO_2-C d^{-1} g^{-1} biomass), respectively. The maximum biomass N-to-total N ratio was observed at the Calliandra site in combination with maximum AEC, qCO_2, ergosterol-to-biomass C ratio, and a minimum biomass C-to-N ratio. The maximum biomass C-to-soil C ratio was measured in combination with maximum biomass C-to-N ratio and minimum qCO_2 at the Teak site, a site with relatively low levels of microbial biomass and activity indices (Table 2). This was even more true for the Gliricidia site where maximum ATP-to-biomass C ratio was found in combination, with minimum ratios of biomass C-to-soil C, biomass N-to-total N, and ergosterol-to-biomass C.

Soil pH, clay content and cation exchange capacity did not affect significantly the different ratios of microbial and organic matter properties in nearly all cases. The only exception was the ATP-to-biomass C ratio revealing significant negative effects of the clay content. The different ratios of microbial and organic matter properties were not significantly interrelated in many cases. The soil C-to-N ratio showed a significant positive correlation with the biomass N-to-total N ratio and a negative with the ATP-to-biomass C ratio. However, the strongest relationship was found between the ratios of biomass N-to-total N and the ergosterol-to-biomass C, followed by the that of biomass C-to-soil C and biomass C-to-N. Also a significant, but negative correlation revealed the biomass C-to-N ratio and the qCO_2. The AEC was positively correlated with the qCO_2 and the biomass N-to-total N ratio with similar levels of significance.

DISCUSSION

Soil organic matter and microbial properties are not affected by small-scale variability of the parent material as reflected by the absence of significant correlations of soil pH, clay content and cation exchange capacity on these indices. Not much experience exist in evaluating the effects of small-scale variability, e.g. of the clay content on biological features in field observations. If biological properties of different climatic regions are compared, clay effects seem to be negligible (Insam *et al.,* 1989; Wardle, 1998). Other investigations found significant clay effects on the landscape scale (Hoeper *et al.,* 1997; Bauhus and Khanna, 1989) and also in laboratory experiments (Filip, 1979). However, the differences in soil organic matter accumulation and soil microbiological properties between the present six sites must be caused by differences in the amount and quality of the present substrate input, i.e. the amount of easily available substrate entering the soil (Anderson and Domsch, 1989; Haron *et al.,* 1998). At those sites with recent transformation into the actual vegetation, especially at the Grevillea site, which used as grassland until 18 months before sampling, effects of former vegetation cannot be excluded. Microclimatic effects of south-facing, north-facing, or the density of the vegetation cover may contribute also the variability between the microbial and soil organic matter properties of the six sites, which exhibit all a considerable small-scale variability. For this reason, the number of replicate sampling points should be increased in future investigations

Although all soil organic matter and microbial properties are within the range described in the literature (Joergensen *et al.,* 1995; Bauhus *et al.,* 1998; Bauhus and Khanna, 1999), the A-horizon of our six forest sites reveals several interesting and important differences. The fist and most important difference to be discussed here is the observation that the biomass C-to-N ratio was larger than the soil C-to-N ratio. This has never been described for another set of soils (Smith and Paul, 1990; Joergensen *et al.,* 1995; Bauhus and Khanna, 1999). The biomass C-to-N ratio increased with decreasing soil C-to-N ratio. In A-horizons, lower C-to-N ratios indicate a higher degree of microbial degradation of soil organic matter or, as in our soils, a smaller percentage of fresh plant material in comparison to those soils with a higher C-to-N ratio. This means probably that the C-input by present vegetation is positively correlated with the actual soil C-to-N ratio. However, no data of the C-input in form of roots and leaf litter are available at the moment for University Forest of Bacnotan. This widely spread gap of knowledge should be closed as soon as possible.

A large biomass C-to-N ratio in soils with a low soil C-to-N ratio suggests that N containing components such as peptides are inaccessible to soil microorganisms and their enzymes. For this reason the biomass N-to-total N ratio seems to be a much better indicator for the availability of substrate to soil microorganisms than the biomass C-to-soil C ratio usually referred as soil organic matter availability index (Anderson and Domsch, 1986; Joergensen and Scheu, 1999). It is unknown to what extent nutrient deficiency adds to the poor availability of N components. The soils of Bacnotan are suspected to have a strong P fixation capacity. P fixation is often combined with an increased anion exchange capacity leading to strong covalent bonding

of soil organic matter (Oades, 1995; Zech *et al.,* 1997). In the volcanic ash soils of Nicaragua, the low P availability to microorganisms results in extremely large biomass C-to-P ratios exceeding the soil C-to-P ratios markedly (Joergensen and Castillo, 2000).

Ergosterol has been demonstrated to be an important indicator of fungal biomass in soil (Montgomery *et al.,* 2000). The ergosterol-to-biomass C ratio was very low in our Bacnotan soils, even lower than the 0.31% observed by Joergensen and Castillo (2000) in arable soils of Nicaragua. Djajakirana et al. (1996) found an average ergosterol-to-biomass C ratio of 0.72% in the A-horizon (0-10 cm depth) of 30 German deciduous forest soils. In forest litter layers dominated by fungi, the ergosterol-to-biomass C ratio is able to exceed 3% (Smolander *et al.,* 1994). In our Bacnotan forest soils, fungi represents 14% of total microbial biomass C if ergosterol is recalculated into fungal biomass C by multiplication by 90 (Djajakirana *et al.,* 1996). In 40 German forest soils, Blagodatskaya and Anderson (1998) estimated by the selective inhibition technique that fungi represents 74 to 94% of total biomass C. Joergensen et al. (1995) suggested that large biomass C-to-N ratios are caused by increased fungi-to-biomass ratios. However, no relationships were found between the ergosterol-to-biomass C ratio and the biomass C-to-N ratio. The significant relationship of the ergosterol-to-biomass C ratio and the biomass N-to-total N ratio suggests that fungi have better facilities to decompose recalcitrant N components than bacteria. No relationship was found between the ergosterol-to-biomass C ratio and the qCO_2 contrasting the results of Sakamoto and Oba (1994) and Blagodatskaya and Anderson (1998) who observed a decrease in qCO_2 with increasing fungal presence.

Not only the ergosterol-to-biomass C ratio is very low, but also the ATP-to-biomass C ratio is very low. Contin et al. (2000), who reviewed only ATP results by the enzymatic luciferin/luciferase system, found an average ratio of 11.7 µmol ± 2.7 (standard deviation; n = 89) ATP g^{-1} biomass C, which is in accordance to the results published by Jenkinson (1988) and Joergensen et al. (1990). This is nearly three times the ratio found in our Bacnotan forest soils. Mao et al. (1992) measured an ATP-to-biomass C ratio of 7.5 µmol g^{-1} in lateritic soils at a pH (H_2O) of between 4.2 and 5.1 under secondary forest in tropical South China. However, they used the SIR method, which is known to underestimate biomass C in strongly acidic soils (Anderson and Joergensen, 1997). The negative relationship between the ATP-to-biomass C ratio and the clay content may indicate insufficient extraction efficiency. However, Friedel (1991), Martens (1995) as well as Dyckmans and Raubuch (1997) did not find any clay effect using the same or similar extraction procedures as used for our Bacnotan forest soils. This is in accordance with the results of Chander et al. (2000 a/b) measuring ATP-to-biomass C ratios in the range reviewed by Contin et al. (2000). If the ATP would be related to biomass N and not to biomass C, the ATP-to-biomass N ratio would be in the normal range assuming that most of the soils reviewed by Contin et al. (2000) had an average biomass C-to-N ratio 6.7 as suggested by Jenkinson (1988). The significant negative correlation between the biomass C-to-N and the ATP-to-biomass C ratio points to the importance of intracellular protein for maintaining high ATP concentrations in the microbial biomass.

Also the AEC values are relatively low compared to other investigations using the same HPLC methods (Chander *et al.,* 2000 a/b) or enzymatic methods (Brookes *et al.,* 1987). However, also the AEC of our set of soils is surprisingly high considering the availability of energy to soil microorganisms. Those bacteria and fungi with high metabolic activity have also high AEC levels as suggested by the highly significant positive correlation between qCO_2 and AEC. This relationship was sometimes (Chander *et al.,* 2000 a), but not always (Chander *et al.,* 2000 b) observed indicating that still more work is necessary to elucidate the meaning of high AEC levels in soils (De Nobili *et al.,* 2000). The markedly higher precision of the HPLC method in comparison to the enzymatic approach raises the chance for improving our knowledge in this important aspect of soil microbial ecology.