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Microbial C, N and P along a weeding regime in a valley cultivation system of northeast India

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Abstract: Soil nutrients and microbial C, N and P were determined at two soil depths (0-15 and 15-30 cm) in no-input, traditionally weeded and unweeded wet-paddy fields in the humid tropics of Arunachal Pradesh, northeastern India. Soil nutrients and microbial C, N and P were greater in the top 0-15 cm soil layer. Microbial C increased after ploughing during late March and decreased during rainy season. The contribution of microbial nutrients to the soil nutrient pool was in the following order: Microbial P > Microbial C > Microbial N. Lowest microbial P values were recorded during crop harvest. Negative correlation between microbial N and soil inorganic N envisage conservation of mineral N within microbial biomass. Soil organic C and microbial C were greater in traditionally weeded paddy fields. This suggests that microbial biomass had a significant role to play in conservation of soil N and P in the stressed ecosystems such as unweeded and moderately weeded paddy fields. This is also substantiated by the greater concentration of microbial N to soil total N and microbial P to soil P.

Resumen: Los nutrientes del suelo y el C, el N y el P microbianos fueron determinados a dos profundidades de suelo (0-15 y 15-30 cm) en arrozales húmedos, uno sin suministros y deshierbado a la manera tradicional y otro no deshierbado, en la región tropical húmeda de Arunachal Pradesh, nordeste de la India. Los nutrientes del suelo y el C, N y P microbianos fueron mayores en la capa superficial de suelo de 0-15 cm. El C microbiano aumentó después del arado durante finales de marzo y decreció durante la época de lluvias. La contribución de los nutrientes microbianos a la reserva de nutrientes del suelo se dio en el siguiente orden: P microbiano > C Microbiano > N Microbiano. Los valores más bajos de P microbiano fueron registrados durante la cosecha del cultivo. Una correlación negativa entre el N microbiano y el N inorgánico del suelo prevé la conservación de N mineral dentro de la biomasa microbiana. El C orgánico del suelo y el C microbiano fueron mayores en los arrozales con deshierbe tradicional. Esto sugiere que la biomasa microbiana juega un papel importante en la conservación del N y del P del suelo en ecosistemas estresados tales como los arrozales no deshierbados y los moderadamente deshierbados. Esto también está apoyado por la mayor concentración de N microbiano en el N total del suelo y del P microbiano en el P del suelo.

Resumo: Os nutrientes e os teores de C, N e P microbianos foram determinados em duas profundidades (0 – 15 e 15 – 30 cm), num solo de arrozal irrigado, sem inputs, tradicionalmente mondado, não mondado, nos trópicos húmidos de Arunachal Pradesh no nordeste da Índia. Os nutrientes do solo e o C, N e P microbianos eram mais elevados na camada superior dos 0 – 15 cm do solo. O C microbiano aumentou depois da lavoura durante o fim de Março, e decresceu durante a estação chuvosa. A contribuição dos nutrientes microbianos para o conjunto dos nutrientes do solo foi da seguinte ordem: P microbi-

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ano > C microbiano > N microbiano. O valor mais baixo do P microbiano foi registado durante a colheita. A correlação negativa entre o N microbiano e o N inorgânico do solo sugere a conservação do N mineral pela biomassa microbiana. O C orgânico do solo e o C microbiano eram mais elevados nos arrozais tradicionalmente mondados. Isto sugere que a biomassa microbiana joga um papel significativo na conservação do N e P do solo em ecossistemas sujeitos a forte pressão tais como os não mondados ou moderadamente mondados. Esta asserção é consubstanciada por uma elevada concentração em N microbiano no N total e do P microbiano no P total.

Key words: Humid tropics, microbial biomass, paddy, soil nutrient pool, valley cultivation, weeds.

Introduction

Although shifting cultivation (locally called 'jhum') is most prevalent in the humid tropics of Arunachal Pradesh, small farmer prefer paddy cultivation in rain fed valley lands. In valley land cultivation either there is no input of fertilizer or farmyard manure, and weeding is partial or traditional. Farmers bury about 20% of the weed biomass traditionally in the field (Ramakrishnan 1992) as a measure of nutrient management in this ecologically fragile agro-ecosystem. This strategy gave us impetus to investigate the role of weeds in soil nutrient management. We also observed variations in retention of weed biomass in the field due to different weeding regimes. Therefore, it was hypothesized that nutrient immobilization in microbial biomass may decrease along an increasing weed density gradient. This contention is based on the general observation that weeds out-compete crops in nutrient uptake at least at the juvenile stage of crop growth (Pandey *et al.* 1971) and therefore, may deplete the soil nutrient pool upon which the soil microorganisms survive. Although, several studies have concentrated on soil nutrient dynamics of shifting agricultural systems in the north-east region (Ramakrishnan 1992), only a few studies have been undertaken to study the role of microbial biomass in soil organic matter and nutrient dynamics, and in relation to weed regime in particular. In this paper, we have tested this hypothesis by studying soil microbial biomass along a weeding regime in rain fed paddy fields at a lower altitude (126 m asl) of Arunachal Pradesh, northeast India. This study could give insights into crop residue management in these low-input systems to maintain soil fertility and crop production.

Materials and methods

The study was conducted at Nirjuli in Kimin-Doimukh block of Papumpare district in Arunachal Pradesh (latitude 28° 21′ N, longitude 94° 21′ E, altitude 126 m asl), northeastern India. The area experiences three main seasons: winter (November-February), dry summer (March-mid May) and wet summer (mid May-October). The mean maximum temperature (34.8°C) during the study year (1999) was recorded in August and mean minimum (13°C) was in January. The annual rainfall was 1564 mm, with peak during July (662 mm). The soil has been developed from geologically young rocks of the Siwalik formations of the sub-Himalayan type consisting of Neogene molassic sediments.

The study was carried out in three paddy fields (site I, site II and site III) and one nursery plot (site IV). These fields were neither fertilized nor manured. The sites were all located in the floodplains of the Dikrong river basin. The area of the experimental plots, weeding regimes, weed density and sampling periodicity are given Table 1. Fifteen soil samples were collected from 0-15 and 15-30 soil layers using a soil corer (5.5 cm inner diameter) from each site at different intervals and placed in sterile polythene bags. The samples were brought to the laboratory, sieved (2 mm mesh screen) and divided into two parts. One part was used in field moist condition to determine soil pH, moisture content, ammonium-N, avaialble-P and microbial C, N and P and the other part was air-dried for the determination of texture, bulk density, water holding capacity (WHC), organic C, total N and total P. Physico-chemical properties of the soil were determined following standard procedures given in Anderson & Ingram (1993).

Table 1. Community characteristics of weeds and crops in selected paddy fields.

	Study sites			
	I	II	III	IV*
Area of paddy fields (m^2)	7200	5800	2500	250
Crop	Paddy	Paddy	Paddy	Paddy nursery
Weeding density status	High	Moderate	Traditional (low)	Negligible
Samplings	a,b,c,d	a,b,c,d	a,c,d	a,b,c,d
Weeds				
Density (no. m^{-2})	120 ± 25	59 ± 3	21 ± 0.9	2 ± 0.3
Basal area (cm^2 m^{-2})	287 ± 13	110 ± 9	69 ± 2	20 ± 0.12
Biomass (g m^{-2})	89 ± 1.2	57 ± 0.8	28 ± 0.2	0.2 ± 0.02
Crops				
Density (no. m^{-2})	55 ± 9	65 ± 12	71 ± 3	160 ± 9
Basal area (cm^2 m^{-2})	227 ± 23	361 ± 19	401 ± 32	112 ± 12
Biomass (g m^{-2})	48 ± 0.9	113 ± 1.2	153 ± 1.2	60 ± 0.91

a – before ploughing (March); b – after ploughing (April); c – during peak vegetative growth (June-July); d – during harvest (August-September); * juveniles while ready for transplantation.

Microbial biomass C was estimated by chloroform fumigation-incubation procedure given by Jenkinson & Powlson (1976) as modified by Srivastava & Singh (1988). Microbial N and P were extracted following chloroform fumigation-extraction procedures using 0.5 M K_2SO_4 and $NaHCO_3$, respectively (Anderson & Ingram 1993). Correction factors used in microbial C, N and P were: Microbial C/0.45 (Jenkinson & Ladd 1981), Microbial N/0.54 (Brookes *et al.* 1985) and Microbial P/0.40 (Brookes *et al.* 1984). The data presented are the means of five replicate determinations on periodical basis and the data have been expressed on an oven-dry weight basis.

Soil samples were also collected aseptically in sterilized polythene bags from 0-15 and 15-30 cm layer in all the sites and were used for the isolation of bacteria and fungi within 24 hours. Soil bacterial population was estimated by Waksman's (1952) method using the nutrient agar medium at 10^5 dilution. Fungal population was estimated by dilution plate technique (Johnson & Curl 1972) using Martin Rose Bengal agar medium at 10^3 dilution in deionized water. The inoculated Petri-dishes were incubated at 30 ± 1°C for 24 h and at 25 ± 1°C for 5 days for bacteria and fungi, respectively.

In all sites, density and basal area of crop and weeds were determined during peak rainy season (June-July) in ten 1m x 1m quadrats according to Kershaw (1973). All the weed species were identified in consultation with the local herbaria of State

Table 2. Weed species composition in the study sites. (+ present, – absent)

Species	Sites			
	I	II	III	IV
Ageratum conyzoides	+	+	+	+
Arundinella bengalensis	+	+	–	–
Arundinella mutica	+	+	+	–
Bambusa nana	+	+	–	–
Borreria hispida	+	–	–	–
Brachiaria distachya	+	+	+	–
Carex phacota	+	–	–	–
Cassia tora	+	–	+	–
Cyperus globulus	+	–	–	+
Digitaria adscendus	+		–	+
Eupatorium odoratum	+	+	–	–
Grewia elastica	+	+	+	–
Imperata cylindrica	+	+	–	–
Macranga indica	+	+	–	–
Mikania micrantha	+	+	–	–
Panicum khasianum	+	+	–	–
Panicum maximum	+	–	+	–
Phyllostachys assamica	–	–	–	+
Saccharum spontaneum	+	+	–	–
Sonerila maculata	–	–	–	+
Zahneria umbellata	–	+	+	–
Total no. of species	18	13	7	5

Table 3. Some soil physical properties, pH and available nutrient concentrations in the paddy fields at two soil depths (0-15 and 15-30 cm).

Parameters	Site/Soil depth (cm)							
	I		II		III		IV	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
Bulk density (g cm^{-3})	1.23	1.32	1.18	1.22	0.95	1.18	1.45	1.25
WHC (%)	48.33	45.33	47.71	46.45	51.41	43.82	41.27	47.62
SMC (%)	24.89	23.15	24.54	22.95	29.75	24.22	20.96	19.74
pH (1:2.5 w/v H_2O)	5.87	5.85	5.53	5.60	5.97	6.08	5.86	5.95
Inorganic N (μg g^{-1})	2.07 (1.40)	1.77 (1.36)	2.35 (0.85)	2.22 (0.72)	2.80 (0.86)	2.65 (0.86)	2.73 (0.66)	3.68 (1.19)
Available P (μg g^{-1})	3.71	2.64	2.53	2.12	1.56	1.65	3.17	2.18

WHC – Water holding capacity; SMC – Soil moisture content
Values in parentheses are the percentages of ammonium-N to inorganic N

Table 4. Soil nutrient concentrations at two soil depths (0-15 and 15-30 cm) in paddy fields.

Site	Organic C (%)		Total N (%)		Total P (μg g^{-1})		Soil C/N	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
I	0.54	0.52	0.22	0.19	18.12	16.83	2.7	2.7
II	0.59	0.46	0.18	0.16	16.32	17.12	3.3	2.9
III	0.64	0.60	0.20	0.21	16.81	15.98	3.2	2.9
IV	0.66	0.51	0.17	0.16	18.57	15.38	3.9	3.2
LSD	0.04	0.05	0.02	0.02	0.92	0.69	0.42	0.18

* LSD at $P < 0.05$; Values are the means of the periodical samplings during the cropping phase.

Forest Research Institute and Botanical Survey of India, Itanagar, Arunachal Pradesh. Nomenclature of plant species followed Hooker (1872-1879). In total there were 21 weed species recorded from the paddy fields across a weed regime. In the traditionally weeded site, there were only 7 weed species, which was slightly higher than the low-weed density nursery plot (Table 2). The high weed density field had 18 weed species, however. For the estimation of biomass, all plants in a randomly located three quadrats (1m x 1m) were uprooted individually and gently washed to remove any adhered soil particles, especially to the root system. The plants were sorted into weeds and crops and oven-dried at 80°C for 48 h and weighed. This, however, could be an underestimation of biomass.

Table 5. Microbial population and biomass at two soil depths (0-15 and 15-30 cm) in paddy fields along a weed regime.

Sites	Population			
	Bacteria*		Fungi**	
	0-15	15-30	0-15	15-30
I	6.32	4.11	13.29	10.22
II	9.40	8.18	29.32	28.39
III	11.32	10.11	40.57	41.38
IV	14.32	12.39	71.31	69.48
LSD***	2.91	3.04	21.22	21.59

* Values are multiples of 10^5 per gram dry soil
** Values are multiples of 10^3 per gram dry soil
*** LSD at $P < 0.05$; Values are the means of periodical samplings during the cropping phase

Linear regressions were used to study the relationships among microbial biomass C, N and P and also the influence of soil and plant community characteristics on soil microbial biomass. LSD at 0.05 level was calculated according to Zar (1974) to determine the variations in different parameters studied across the weeding regime.

Results and discussion

The species composition of weeds, their density and basal area are given in Tables 1 and 2. Weed as well as basal cover was highest at site I followed in descending order by site II, III and nursery plot. Weed biomass was closely correlated with their

Table 6. Microbial biomass at two soil depths (0-15 and 15-30 cm) in paddy fields along a weed regime.

Sites	Biomass (μg g^{-1})							
	C		N		P		Microbial	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
I	175.21 (3.2)	157.41 (3.0)	40.63 (1.95)	57.54 (2.0)	4.3 (23.7)	3.91 (23.2)	4.3	2.7
II	196.62 (3.3)	169.26 (3.7)	23.27 (1.3)	21.61 (1.4)	4.81 (29.5)	4.53 (26.5)	8.5	7.8
III	200.04 (3.1)	173.12 (2.9)	11.77 (0.59)	11.96 (0.57)	3.86 (22.9)	3.14 (19.7)	16.9	14.5
IV	170.08 (2.58)	154.88 (3.03)	12.78 (0.75)	11.63 (0.73)	4.46 (24.0)	4.37 (28.4)	13.3	13.3
LSD***	15.04	15.28	11.60	10.52	4.07	0.54	4.8	4.7

***LSD at $P < 0.05$; Values are the means of periodical samplings during the cropping phase.
Values in parentheses are percentage contribution of microbial biomass to respective soil nutrients.

density and basal area (r = 0.984 – 0.986). Crop density was much greater in the nursery plots because of transplantation of crop seedlings. Weed density was negatively correlated with crop density (r = -0.722). In crops, density and basal area were negatively correlated (r = -0.932).

The soil was a sandy loam with 41-51% water holding capacity. Soil moisture content (SMC) was greater in the top 0-15 cm soil layer (Table 3). Higher concentration of soil nutrients (Tables 3 & 4) and microbial population (Table 5) and biomass (Table 6) in the top 0-15 cm soil layer as compared to the sub-soil layer (15-30 cm) have been attributed to the greater accumulation of organic matter on the surface soil, and relatively better microbial activity (Arunachalam *et al.* 1999). Along a temporal scale, microbial C increased significantly following manual, moderate ploughing (25-30 cm till) in late March in all sites (Fig. 1). Reduction in microbial C during monsoon (June) was mainly due to the loss of microbial propagules that might have reduced the biomass (Maithani *et al.* 1996). In high

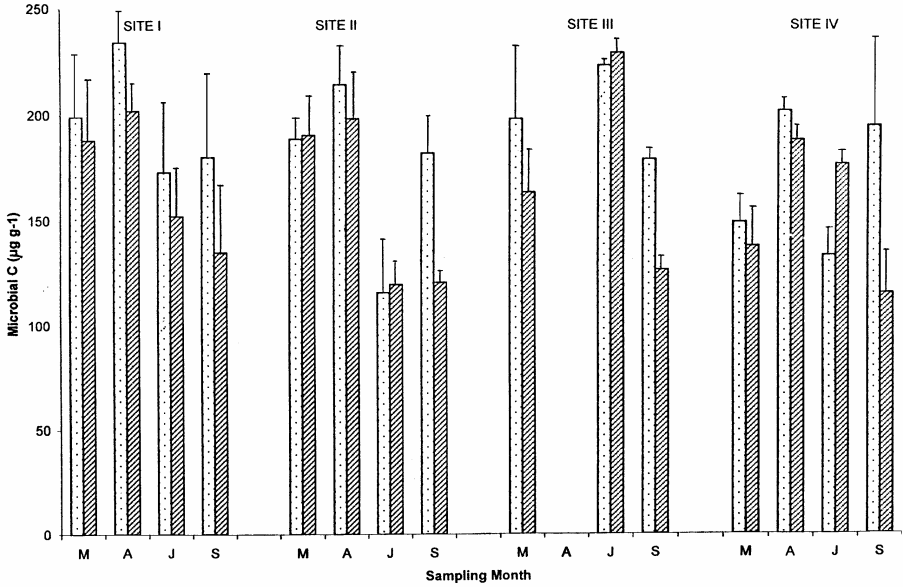

Fig. 1. Temporal variations in microbial C (μg g^{-1}) during cropping phase. Samples of site III during April could not be collected due to unforeseen circumstances. ☐ 0-15 cm and ☐ 15-30 cm soil depths.

weed density field (site I), microbial N declined following ploughing but gradually increased during monsoon (Fig. 2), emphasizing the role of microbial biomass in N retention during stressed conditions (e.g. high rainfall, leaching and runoff loss of nutrients) (Zogg *et al.* 2000). Microbial P, in general, decreased gradually through the cropping phase and lowest values were recorded during harvest in September (Fig. 3). Also, our results show that most of soil P is retained within microbial biomass (20-30%). Although P is a relatively immobile element compared to N, its deficiency could add to soil chemical constraints that are related to soil acidity (Sanchez & Salinas 1981). A significant negative correlation between soil pH and microbial P was also observed in this study ($r = -0.542$, $P < 0.01$, $df = 29$). The values obtained for microbial P (3.1 – 4.8 μg g^{-1}) was, however, extremely low as compared with the reported values for grasslands and woodlands (4.9 – 67.2 μg g^{-1}; Brookes *et al.* 1984). Comparing the values of microbial biomass in properly scientifically managed paddy fields (C – 232 μg g^{-1}, N – 32 μg g^{-1}, P – 11 μg g^{-1}; Goswami *et al.* 2000), the present values were slightly lower, but comparable to a shifting agricultural field (C – 204 μg g^{-1}, N – 22 μg g^{-1}, P – 9.5 μg g^{-1}; Goswami *et al.* 2000).

Above discussions suggest relatively low rates of nutrient immobilization in microbial biomass. Evidently, the recorded microbial N values were very low as compared to several reported values (32 – 242 μg g^{-1}; Goswami *et al.* 2000). The contribution of microbial C to soil organic C was also lower but well within the reported range (1.5 – 5.3%) for tropical soils (Luizao *et al.* 1992). Nonetheless, microbial P contributed more to the soil total P (19.7 – 29.5%) which is very high compared to other arable lands (1.4 – 3.5%) and grasslands (2.0 – 4.3%) elsewhere (Brookes *et al.* 1984).

In terms of contribution of microbial biomass to soil nutrient pool, the trend is: microbial P > microbial C > microbial N. This indicates the limiting nature of N in the tropical soils. The amounts of available N (ammonium N in particular) measured in this study are extremely low when compared to our earlier studies with natural ecosystems in this region (Arunachalam *et al.* 1999). Saxena & Ramakrishnan (1986) reported a strong positive correlation between soil organic C and total N, as the rate of N transformation may be quite rapid. In this study, we did observe such a relationship, but was weak ($r = 0.397$). This indicates that (i) the soil organic C level in these paddy fields is not stable particularly during the cropping phase, and (ii) the conversion of total N to ammonium and nitrate is relatively slow.

Several studies have established close correlations between soil organic matter and microbial

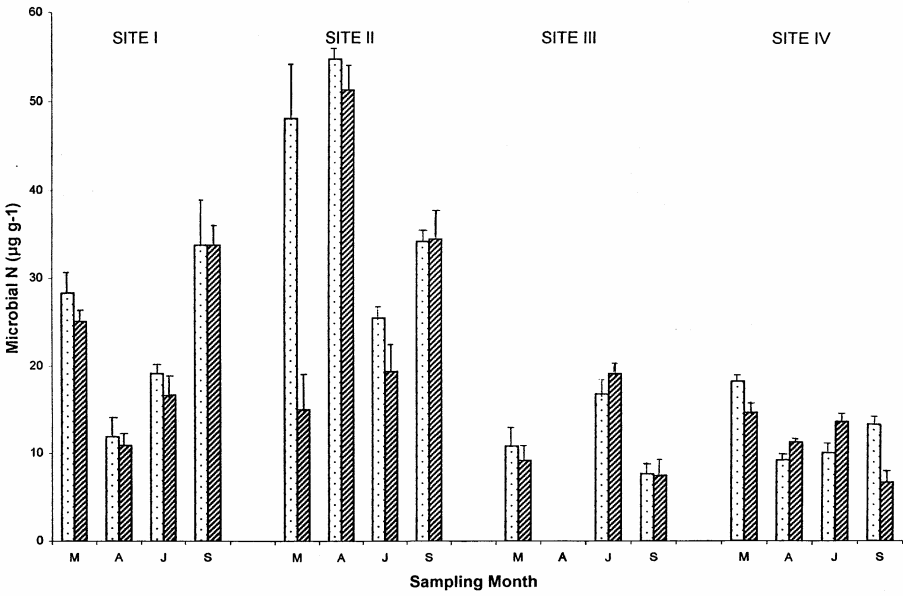

Fig. 2. Temporal variations in microbial N (μg g^{-1}) during cropping phase; ☐ 0-15 cm and ☐ 15-30 cm soil depths.

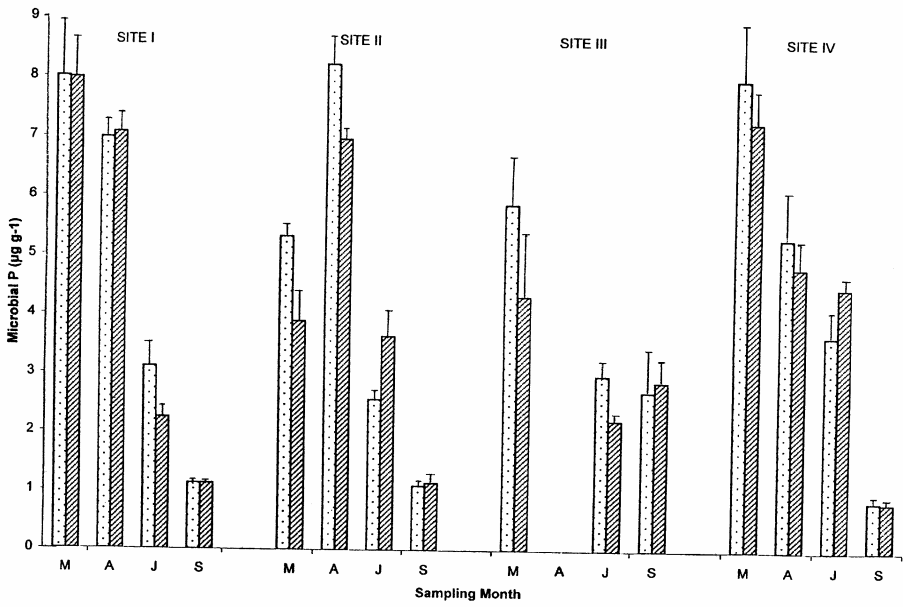

Fig. 3. Temporal variations in microbial P (µg g^{-1}) during cropping phase; ☐ 0-15 cm and ☐ 15-30 cm soil depths.

biomass and also among microbial C, N and P. In the studied systems, microbial C, N and P were not found to be related to total soil C and N. The wide C/N ratio in the study sites could be due to recent organic inputs. However, Bremner & van Kessel (1992) attributed a wider C/N ratio to a greater microbial activity.

Microbial C and P were not influenced by the growth of weeds. Nevertheless, microbial N showed a strong positive correlation ($P < 0.01$) with weed density ($r = 0.983$), its basal area ($r = 0.962$) and biomass ($r = 0.937$). It has always been debated whether plants or microorganisms are the superior competitors for soil N, a primary limiting nutrient in terrestrial ecosystems. Traditionally, it was assumed that plants could access only the inorganic form of N made available via the mineralization of soil organic matter (Jackson *et al.* 1989). It was also assumed that microorganisms are the superior competitors for this, because of their major role in the mineralization process (Stark & Hart 1997). Although, it is difficult to assess the direct competition between plants and microorganisms for soil N owing to multiple and complex pathways in the N cycle, most organisms are usually limited by the supply of available organic C.

Jackson *et al.* (1989) reported that within a short time scale, soil microbes do compete better than plants for N, particularly ammonium-N: its uptake by microbes was 5-times faster than that by plants. The nitrate–N uptake rate by microbes was double that of plants. The negative correlation between microbial N and nitrate–N ($r = -0.410$, $P < 0.05$, $df = 29$) envisage the conservation of this form of mineral–N within microbial biomass, particularly under high weed density conditions. Though not substantiated, it could be said that the low level of ammonium-N in the soil as compared to nitrate-N may be due to comparatively faster nitrification rates. This, however, remain tentative, as we did not study the N mineralization pattern in relation to nitrifier population that perhaps, may have explained the varying levels of mineral-N forms in the soil during the cropping phase. Thus, in agroecosystems where the farmer grows short life-cycled crops for immediate crop production, microbial biomass due to rapid turnover of microbial cells, plays a crucial role in N release and cycling. Therefore, microbial N could be used as an indicator of N-flux in agricultural systems, especially under stressed conditions (Zogg *et al.* 2000).

Conclusions

The reduction in microbial N with decreasing weed density indicates that the microbial biomass releases N that would help in increased availabil-

ity of mineral-N. Eventually, the growth, biomass and productivity of the crops are predictably better in low-weed density fields or the traditionally weeded systems. Whereas, the higher microbial N in high-weed density fields indicates greater N immobilization, which could be a nutrient conservation mechanism to help replenish the soil N over a long-term for sustaining the crop production.

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