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Effect of land management on soil microbial N supply to crop N uptake in a dry tropical cropland in Tanzania

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ABSTRACT

In Sub-Saharan Africa, conservation of available soil N during early crop growth, when N loss by leaching generally occurs, is important to improve crop productivity. In a dry tropical cropland in Tanzania, we assessed the potential role of soil microbes as a temporal N sink-source to conserve the available soil N until later crop growth, which generally requires substantial crop N uptake. We evaluated the effect of land management [i.e., no input, plant residue application before planting (P plot) with or without fertilizer application, fertilizer application alone, and non-cultivated plots] on the relationship between soil N pool [microbial biomass N (MBN) and inorganic N] and crop N uptake throughout the ~120-d crop growth period in two consecutive years. In the P plot, MBN clearly increased (~14.6–29.6 kg N ha⁻¹) early in the crop growth period in both years because of immobilization of potentially leachable N, and it conserved a larger soil N pool (~10.5–21.2 kg N ha⁻¹) than in the control plot. Especially in one year in which N leaching was critical, increased MBN maintained a larger soil N pool in the P plot throughout the experimental period, and a delay of increased MB C:N ratio and a substantial decrease in MBN was observed, indicating better soil microbial N supply for crop N uptake during later crop growth. Therefore, plant residue application before planting should enhance the role of soil microbes as a temporal N sink-source, leading to the conservation of potentially leachable N until later phase of crop growth, especially in years in which N leaching is relatively severe. Although further studies are

necessary, our results suggest that plant residue application before planting is a promising option to achieve better N synchronization.

1. Introduction

45 In Sub-Saharan Africa, soil nitrogen (N) is the most limiting factor for crop
production because of low soil fertility and the inability of small farms to afford
fertilizers (Bationo and Buerkert, 2001; Palm et al., 2001). Thus, to improve crop
productivity in this region, it is necessary to improve the synchronization of soil N
supply and crop N uptake by reducing N loss (Nyamangara et al., 2003; Adeboye et al.,
50 2006; Gentile et al., 2009). Distinct net N mineralization generally occurs early in the
rainy season during early crop growth in dry tropical croplands, because organic matter
(OM) with a low C:N ratio accumulates during the dry season (Kushwaha et al., 2000;
Singh et al., 2007a). However, heavy rainfall often leaches out mineralized N during
early crop growth, resulting in depletion of available soil N during later crop growth
55 when the need for N uptake is greatest (Hartemink et al., 2000; Chikowo et al., 2004;
Shahandeh et al., 2004). Hence, efficient use of soil N supply in this agroecosystem
requires conservation of available soil N at the surface layer during early crop growth
and increased soil N supply during later crop growth.

Soil microbes play an important role as an N sink-source in dry tropical
60 agroecosystems (Singh et al., 1989; Singh et al., 2007b; Sugihara et al., 2010a, b). In this
agroecosystem, soil microbe populations (and consequently microbial biomass N; MBN)
generally increase and remain high during the dry season, thus acting as an N sink,
whereas soil microbes decrease during the rainy (crop) season, thus acting as an N

source (Singh et al., 1989; Sugihara et al., 2010a). In addition, Singh et al. (2007b)
65 observed a clear negative relationship between crop root biomass and MBN at the
grain-forming stage in dry tropical India, suggesting that MBN acts as an N source for
crop growth. Similarly, our previous study found decreased MBN and an increased MB
C:N ratio in relation to the crop N uptake pattern at the grain-forming stage, whereas
these soil microbial dynamics were not observed in a non-cultivated plot in dry tropical
70 Tanzania (Sugihara et al., 2010b). Therefore, soil microbes likely act as a soil N source
during later crop growth in dry tropical croplands.

Generally, land management, especially OM application (i.e., C substrate
input), affects soil microbial dynamics (Srivastava and Lal, 1994; Petersen et al., 2003;
Spedding et al., 2004; Chikowo et al., 2006). Previous studies reported that C input
75 temporarily increases MBN by immobilizing soil inorganic N, suggesting that soil
microbes may constitute an ephemeral N sink that conserves potentially leachable N at
the surface layer (Friedel et al., 2001; Herai et al., 2006; Mtambanengwe and Mapfumo,
2006). However, our previous study showed that OM application did not significantly
affect the MBN dynamics, possibly because of the timing (8 months before planting) and
80 small amount (a single application of 2.5 Mg C ha⁻¹) of application. Based on these
results, we hypothesize that OM application before planting in a dry tropical cropland
enables soil microbes to immobilize the potentially leachable N during early crop
growth, leading to increased soil microbial N supply for crop N uptake later during the

growing season. However, little is known about the effect of OM application before
85 planting on the time-course of N partitioning between the soil N pool (MBN and
inorganic N) and crop N uptake during crop growth in dry tropical agroecosystems.
Therefore, our objectives in this study were to evaluate the effect of OM application
before planting on soil-crop N dynamics and to ascertain whether OM application before
planting improves soil microbial N supply for crop N uptake, especially later in the
90 growing season, in a dry tropical cropland in Tanzania.

2. Materials and methods

2.1. Description of the study site

The field experiment was conducted from March to June or July in 2007 and
95 2008 at the Agricultural Experimental Station, Sokoine University of Agriculture,
Morogoro, Tanzania, located at 6°, 51' S and 37°, 4' E at an elevation of 579 m (Sugihara
et al., 2010b). The mean annual temperature was 24.5°C (2000–2005), and the annual
rainfall was 750–1000 mm. The rainy season usually exhibited a bimodal distribution.
The long rainy season from mid-February to May was more reliable with better rain
100 distribution for cropping, whereas the duration/intensity of the short rainy season from
October to December was less predictable. The study was conducted during the long
rainy season. The soil at the experimental site is Kanhaplic Haplustults (Soil Survey
Staff, 2006). Briefly, soil characteristics of the plow layer (0–15 cm) were as follows: pH

5.8, as determined in water (soil:water, 1:5 w/v); sandy clay soil texture containing
105 56.0% sand, 10.9% silt, and 33.1% clay; bulk density, 1.21 g cm⁻³; total organic C and N,
12.4 and 1.1 g kg⁻¹ soil, respectively.

2.2. Experimental design

The experimental design included the following five treatments:

- 110 (1) C plot = control (no input);
- (2) F plot = chemical fertilizer-treated plot (urea equivalent to 100 kg N ha⁻¹ and
triple super phosphate equivalent to 50 kg P ha⁻¹);
- (3) P plot = plant residue-treated plot (explained below);
- (4) PF plot = plant residue- and chemical fertilizer-treated plot;
- 115 (5) B plot = bare plot (without maize plants; the soil surface was kept bare of all
plants including weeds during the experimental period). The non-maize
cultivation plot (B plot) was treated as a comparable plot, to clearly evaluate
the effect of crop N uptake on MBN and inorganic N variations.

In P and PF plots, plant residue was applied as follows: maize straw (C:N =
120 ~110) and leaves (C:N = ~40) were chopped into 10-cm pieces and incorporated into the
soil (15 cm depth) using hand hoes. In the current study, the application of plant residue
was considered the C substrate, which should stimulate soil microbial immobilization of
potentially leachable N during early crop growth. To prevent severe N competition

between soil microbes and crops due to excessive C input (Vigil and Kissel, 1991; Hadas
125 et al., 2004), 2.5 Mg C ha⁻¹ (35 kg N ha⁻¹) plant residue was applied before seeding
(March), which is equivalent to mature maize biomass (without cobs) and is also
equivalent to ~50% of decomposed C during the crop growth period (Sugihara et al.,
2010a, b). The plant residue was applied after the first heavy rainfall event in March,
when Tanzanian farmers generally begin to cultivate. To avoid severe N depletion after
130 excessive C input, seeding was conducted at least 2 weeks after plant residue
application and in concert with a rainfall event. After harvest (July), 5.0 Mg C ha⁻¹ (70
kg N ha⁻¹) plant residue was also applied to increase soil OM, thereby enhancing the
ability of soil microbes to assimilate soil N. Farmers in this region generally burn the
remaining plant residues after harvest (July or August) or before cultivation (February
135 or March). Chemical fertilizer in the F and PF plots was applied as follows: urea was
broadcast separately, 35 kg N ha⁻¹ at 7 days after planting (DAP) and 65 kg N ha⁻¹ at 35
DAP for F and PF plots, and triple super phosphate was broadcast at the time of
seeding.

Each experimental plot (8 × 8 m) was laid down in a randomized block design
140 using three replicate plots per treatment, with an unplanted >1 m strip separating each
block. Two maize (*Zea mays* L. var. *Staha*) seeds were planted per hole at a spacing of
80 × 30 cm on March 15, 2007 and March 23, 2008 (i.e., 0 DAP), and were thinned to one
plant per hole at 14 DAP. We replanted on March 28, 2007 (Fig. 1) because small

animals dug out the planted seeds. This replanting did not significantly affect crop
145 growth in 2007 (see Results). All treatment plots were weeded at 15 and 36 DAP, and
additionally at 63 DAP only in the B plot to completely remove grass. All weeded
materials were removed from the plots. The maize was harvested on July 5, 2007 (99
DAP) and July 3, 2008 (102 DAP). No irrigation was applied, and the experiment was
150 maintained under rain-fed cultivation. The same experimental site was used as in our
previous study (Sugihara et al., 2010b) because we had assessed that there was no
remaining influence of previous land management, based on soil chemical and biological
analysis.

2.3. Environmental factors

155 The air and soil temperature at a depth of 5 cm and the volumetric water
content (VWC) in the surface soil (0–15 cm) were monitored hourly for two replications
of the C and PF plots using a data logger system (107 thermistor probes for temperature
and CS616 for soil VWC, connected to a CR-10X data logger; Campbell Scientific, Inc.,
USA). Rainfall was also monitored hourly at the experimental site using the same
160 CR10X data logger system and a TE525MM device (Campbell Scientific, Inc.).

2.4. Soil sampling and analyses

Soil samples (0–15 cm) were collected ten times during each ~120-d crop

growth period. For each sample, six soil cores (2×15 cm) taken within 10 cm of a
165 randomly selected plant inside the plot (7×6 m, avoiding the plot edge) were combined
and mixed for each replication. The matured maize roots of the planted variety in the
current experiment were mostly distributed (74–86%) in the 0–15 cm depth, indicating
the importance of the soil surface (15 cm depth) for crop N uptake (unpublished data).
Soil samples were immediately transported to the lab in a 4°C cooler (~0.5 h), sieved
170 through a 4-mm mesh screen after removing visible plant debris, and stored under
field-moist conditions at 4°C. Samples were taken for determination of gravimetric soil
moisture, inorganic N (NH_4^+ and NO_3^-), MBC, and MBN. Soil bulk density (15 cm
depth) was also determined taking another three soil cores in each plot at the harvest in
2007 and 2008 to calculate the amount of nutrients per unit area (kg N ha^{-1} ; 0–15 cm) in
175 each plot.

Upon sieving, 10.0-g soil samples were weighed into aluminum dishes that
were placed in a 105°C oven for 48 h, after which the dry weight was recorded.
Gravimetric soil moisture was the difference in soil weight before and after oven-drying.
Inorganic N was extracted from 10.0 g soil (dry base) with 30.0 ml of 1 M KCl for 30 min
180 on an orbital shaker, and the suspension was centrifuged and filtered through filter
paper (No. 5C, Advantec, Japan). NH_4^+ and NO_3^- in the extract were determined using
the modified indophenol blue (Rhine et al., 1998) and modified Greissess Ilovay
(Mulvaney, 1996) methods, respectively. MBC and MBN were measured using the

fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Briefly, soil
185 samples (8.0 g dry base) were fumigated with ethanol-free CHCl_3 for 24 h at 25°C. After
removal of the CHCl_3 , soluble C and N were extracted from the fumigated and
non-fumigated samples with 32.0 ml of 0.5 M K_2SO_4 for 30 min on an orbital shaker.
Total organic C and extractable N in the filtered extract were determined using a
TOC-N auto-analyzer (TOC-V carbon analyzer with an IN unit, Shimadzu, Japan).
190 Microbial C flush (difference between extractable C from fumigated and non-fumigated
samples) was converted to MBC using a K_{EC} factor of 0.45 (Vance et al., 1987). Microbial
N flush was also converted to MBN using a K_{EN} factor of 0.54 (Brookes et al., 1985). All
measurements were done in triplicate.

195 *2.5. Microbial respiration as a measure of microbial activity in situ*

Microbial activity is closely linked to soil OM decomposition, which results in N
mineralization or immobilization, and microbial respiration is one of several microbial
activity indices. To evaluate the importance of soil microbes in decomposition during
crop growth, soil respiration was measured in all plots using a closed-chamber system
200 (Sugihara et al., 2010b). As soil respiration consists of plant-root respiration and
microbial respiration, the plant-root respiration was excluded using the trenching
method. Polyvinyl chloride (PVC) cylinders (13 × 30 cm) were inserted into the soil to 15
cm depth, and the enclosed soil was later covered with a fine mesh to support the core

soil sample. The CO₂ efflux rate was measured nine (2007) or ten (2008) times. To block
205 CO₂ evolved from plant-root respiration, for each experiment the PVC cylinder was
removed and the bottom of the cylinder covered with a plastic sheet before replacing the
cylinder into the hole. Gases in the headspace of the PVC cylinder were sampled at 0
and 40 min after the top of the cylinder was covered with a plastic sheet, and the CO₂
efflux rate was calculated based on the increase in CO₂ concentration in the cylinder
210 after 40 min. Gas samples were analyzed with an infrared CO₂ controller (ZFP9AA11;
Fuji Electric, Japan). Five replicate measurements were made for each plot.

2.6. Plant sampling and analysis

Above-ground plant material was sampled seven times in both years on the
215 same day as soil sampling. Six plant samples were collected from 5 × 0.3 m randomly
selected areas in respective plots. The plant material was divided into leaves, stems,
cobs, and grain and dried 1 wk in a glass greenhouse and then 48 h in an 80°C oven, and
the dry matter was weighed. Each sample was ground, and the N content was measured
using a dry combustion method with an NC analyzer (Vario Max CHN, Elementar,
220 Germany). Data are expressed on an area basis (kg N ha⁻¹) by multiplying the N
percentage by the plant biomass per unit area. To estimate crop yields, other maize cobs
were collected from 5 × 1.8 m sections (~30 cobs) per replicate and weighed on the last
sampling day.

225 *2.7. Statistical analyses*

All statistical analyses were performed with SYSTAT 11 (SYSTAT Software, Richmond, CA, USA). All data are expressed on a dry-weight basis. The Tukey's test was used to detect statistically significant differences between treatments in each year. The effects of treatment and sampling time were assessed using repeated-measures
230 analysis of variance (RM-ANOVA). One-way ANOVA was also used to determine the significance of differences between data for several variables (MBC, MBN, MB C:N ratio, CO₂ efflux rate, NH₄⁺, NO₃⁻, crop N, and crop yield) for the whole treatment plots at each sampling time. When ANOVA indicated significant differences, mean comparisons were performed with Tukey's Kramer multiple comparison test. In all cases, $p < 0.05$
235 was considered significant.

3. Results

3.1. Environmental factors

Figure 1 presents the fluctuation of rainfall and VWC (0–15 cm) during the
240 experimental period. The total rainfall was 546.0 mm (2007) and 464.5 mm (2008). In 2007, only 39.9% (217.8 mm) of the total rainfall occurred during the early crop growth period (0–27 DAP), but was 74.2% (344.8 mm) in 2008. In addition, there was substantial and consistent rainfall until 72 DAP in 2007, whereas in 2008 the rainfall

was mostly concentrated from 0–42 DAP with little rainfall thereafter. As a result, a
245 high VWC (approximately –10 kPa) was maintained longer in 2007 (0–67 DAP) than in
2008 (4–35 DAP) (Fig. 1). From the rainfall distribution and VWC variations, the
experimental period could be roughly divided into the wet period (from –25 to 67 DAP in
2007, and from –25 to 35 DAP in 2008) and the relatively dry period (from 68 to 103
DAP in 2007, and from 36 to 103 DAP in 2008). Average air temperature during the
250 experimental period was 23.5°C (2007) and 23.2°C (2008). In the C and PF plots,
respectively, average soil temperature was 27.8°C and 26.8°C (2007) and 27.6°C and
26.2°C (2008). Plant residue application decreased the soil temperature as previously
reported (Tilander and Bonzi, 1997).

255 3.2. Temporal variations in soil microbes (MBC, MBN, and MB C:N ratio)

Figure 2 shows the variations in MBC, MBN, and MB C:N ratio during the
experimental periods in 2007 and 2008. All soil microbial factors fluctuated significantly
during the experimental period in all treatment plots for both years ($p < 0.05$; data not
shown), except for MB C:N ratio in the B plot in both years. Table 2 shows the averaged
260 MBC, MBN, and MB C:N ratio values in 2007 and 2008. According to RM-ANOVA
analysis, MBN in both years increased significantly in both P and PF plots (Table 3).
However, MBC and MBN levels in the P and PF plots at the beginning of the
experiment (March) both in 2007 and 2008 were similar to the C plot, despite the plant

residue applications in July.

265 MBC and MBN in the C, F, and B plots in 2007 gradually decreased and
remained low until 71 DAP (Fig. 2a, b). In contrast, at -13 DAP the P and PF plots
maintained high MBC levels, and MBN increased after plant residue application (-23
DAP), with both gradually decreasing until 43 DAP, although they were relatively
larger than in the C and F plots. In 2008, as in 2007, MBC and MBN in the P and PF
270 plots at -6 DAP increased after plant residue application (-18 DAP), and the
fluctuations in these variables in the P and PF plots were similar to those in the C and F
plots but were often greater than observed in the C plot.

The MB C:N ratio in all cultivated plots was constant until 57 DAP in 2007 and
until 28 DAP in 2008. Later in the crop growth period, the MB C:N ratio in cultivated
275 plots increased significantly at 71 and/or 85 DAP in 2007, and at 42 and/or 56 DAP in
2008, whereas the MB C:N ratio in the B plot did not fluctuate clearly during this period
(Fig. 2c, f). In addition, the timing of the increased MB C:N ratio was clearly delayed in
the P and PF plots compared to the C plot in both years.

280 3.3. Temporal variations in microbial respiration

Figure 3 shows the variations in microbial respiration (CO₂ efflux rate) in 2007
and 2008, and Table 2 presents the averaged CO₂ efflux rate. CO₂ efflux rate fluctuated
substantially during the experimental period in both years ($p < 0.0001$; data not shown).

The CO₂ efflux rate in all treated plots was generally high during the wet period and
285 continuously low during the dry period in both years (except for 42 DAP in 2008) (Fig. 3).
Plant residue, but not fertilizer application, significantly increased the CO₂ efflux rate
(Table 3). Plant residue application substantially promoted microbial decomposition
during both the wet and dry periods. The CO₂ efflux rate correlated strongly with soil
moisture ($p < 0.001$, data not shown) in all treatment plots in both years, and therefore
290 microbial decomposition was substantially greater during the wet period (early crop
growth) and relatively low during the dry period (late crop growth) as observed in our
previous study (Sugihara et al., 2010b).

3.4. Temporal variations in soil inorganic N

295 The variations in soil NO₃⁻ and NH₄⁺ are shown in Figure 4 (2007) and Figure
5 (2008), and Table 2 presents the averaged NO₃⁻ and NH₄⁺ values. In 2007, NO₃⁻ in the
C and P plots increased gradually until 15 DAP and tended to decrease and remain low
after 29 DAP (Fig. 4). At 71 DAP, NO₃⁻ in the P plot increased temporarily (from 23.6 kg
N ha⁻¹ at 57 DAP to 34.0 kg N ha⁻¹ at 71 DAP), although NO₃⁻ in the C plot remained
300 low (22.4 kg N ha⁻¹ at 57 DAP; 23.4 kg N ha⁻¹ at 71 DAP). Inorganic N (NO₃⁻ + NH₄⁺) in
the F and PF plots increased significantly after the first and second fertilizer
applications at 0 and 43 DAP, respectively. Increased inorganic N in the F and PF plots
remained significantly higher than in the C plot until 85 DAP.

In contrast, in 2008, NO_3^- in all treatment plots decreased substantially from 5
305 to 28 DAP (Fig. 5). Inorganic N in the C plot was consistently low after 28 DAP,
although inorganic N in the P plot was relatively higher than in the C plot during the
dry period. NO_3^- in the F and PF plots increased at 42 DAP, which was after the second
fertilizer application (35 DAP), but did not increase after the first fertilizer application
(7 DAP).

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3.5. Crop N uptake and yield

Table 1 presents the crop N uptake pattern and its yield in each plot for both
years. The crop N uptake pattern was generally sigmoid-type, and most crop N uptake
occurred after 42 DAP in both years (Table 1). In the current study, early crop growth
315 was defined as before 42 DAP, and later crop growth as after 43 DAP. Both crop N
uptake and crop yield in the PF plot were greater than in the other plots, owing to the
larger N input (100 kg N ha^{-1} by chemical fertilizer and 105 kg N ha^{-1} by plant residue
application per year). Fertilizer application also significantly increased the crop N
uptake and crop yields in the F plot in both 2007 and 2008 compared to the C and P
320 plots, although crop N uptake in the F plot was similar to that in the P plot in 2008.

In 2007, crop N uptake continued until 71 DAP (C plot) or until 85 DAP (P, F,
and PF plots). In 2008, crop N uptake continued until 70 DAP (C and F plots) or until 87
DAP (P and PF plots). Crop N uptake during 43–57 DAP in 2007 was relatively low in

the P plot compared to the other cultivated plots. Because crop N uptake in the P plot of
325 2007 continued until 85 DAP, total crop N uptake in the P plot was similar to that in the
C plot. In 2008, crop N uptake during 43–56 DAP was substantially lower in all
cultivated plots compared to the same growth period in 2007. As a result, total crop N
uptake in 2008 was substantially less than in 2007. In 2008, total crop N uptake and
crop yield in the P plot were similar to those in the F plot and were slightly greater than
330 in the C plot, but these apparent differences were not significant.

4. Discussion

4.1. Effect of land management on the soil N pool during early crop growth

Critical N leaching in dry tropical croplands occurs especially during early crop
335 growth, when crop N uptake is too small to assimilate much N from the soil (Chikowo et
al., 2004; Mtambanengwe and Mapfumo, 2006). In our present study, a distinct decrease
in the soil N pool was also observed from 5–28 DAP in 2008 (Fig. 5). Because the rainfall
(344.8 mm) was substantially greater than the evapotranspiration (~90–140 mm) and
VWC was kept higher (approximately –10 kPa), there should have been substantial
340 downward movement of water from the surface layer, resulting in critical N leaching in
this period. In contrast, no substantial N leaching occurred during the same period in
2007 owing to few heavy rainfall events.

In both years, plant residue application before planting immediately increased

MBN in the P plot to 13.1~19.5 kg N ha⁻¹ higher than the C and F plots after plant
345 residue application, resulting in a larger or similar soil N pool in the P plot during early
crop growth compared with the C and F plots, respectively. This was because the C:N
ratio of applied plant residue was high (~70), and soil microbes immobilized inorganic N
that could have potentially leached. According to our estimation based on the
relationship between soil moisture and CO₂ efflux rate (Funakawa et al., 2006),
350 decomposed C from -20 to 40 DAP in the P and PF plots was ~750~920 kg C ha⁻¹ higher
than in the C and F plots in 2007 and 2008. This indicates that the C:N ratio of applied
plant residues was gradually reduced during this period so that net N mineralization of
applied plant residue may not occur before 40 DAP in the P and PF plots (Vigil and
Kissel, 1991), when the risk of critical N leaching was high. An increase of NO₃⁻ in the P
355 plot was only observed at 15 DAP in 2007. Because of heavy rainfall at 14 DAP (63.5
mm) after a period of no rain, i.e., 5–14 DAP (Fig. 1), re-wetting of dry soil should cause
net N mineralization by the “priming effect” (Kuzyakov et al., 2000). Thus, plant residue
application before planting enhanced the role of soil microbes as a temporal N sink by
immobilizing the potentially leachable N, leading to conservation of available soil N in
360 the surface soil until later crop growth. Herai et al. (2006) also found that the increase
in MBN upon OM application conserved a larger soil N pool in a Japanese sandy
cropland.

On the other hand, OM application in July did not clearly increase the soil N

pool at the beginning of cultivation in both years, although it provided a substantial
365 amount of N (70 kg N ha^{-1}). This may be attributable to the following: (1) the
decomposition during the dry season and short rainy season was substantial in the P
and PF plots, $\sim 1.3\text{--}1.8 \text{ Mg C ha}^{-1}$ (Sugihara et al., *In press*); (2) soil macro fauna should
decompose applied OM substantially even in the dry season (Mando and Brussard, 1999,
Esse et al., 2001), although these decompositions could not be evaluated by CO_2 efflux
370 measurements; and (3) critical N leaching should have also occurred even in the short
rainy season, because we previously observed heavy rainfall events that were
substantially larger than the evaporation and decreased inorganic soil N in all treated
plots during the short rainy season in each experimental year (Sugihara et al., 2010a).
The third possibility is likely the main reason for the unclear effect of OM application in
375 July on the soil N pool in the subsequent March, although further study is needed.

4.2. Effect of land management on the relationship between soil microbes and crop N uptake during later crop growth

During later crop growth (i.e., dry period) in 2007 and 2008, the soil microbes
380 (MBN and MB C:N ratio) and crop N uptake pattern were closely related, as previously
observed (Sugihara et al., 2010b). The MB C:N ratio increased significantly, and MBN
tended to decrease in all cultivated plots during later crop growth in both years,
possibly because increased crop competitiveness for N driven by an increased root

system enhanced assimilation of the soil N pool (Bottner et al., 1999; Mayer et al., 2003).

385 Many other studies have also found an increased MB C:N ratio and decreased MBN,
indicating the contribution of soil microbial N to crop N uptake at the grain-forming
stage in dry tropical croplands (Ghoshal and Singh, 1995; Kushwaha et al., 2000; Singh
et al., 2007b). Moreover, in the current study, the aforementioned microbial factors in
the B plot did not fluctuate at the grain-forming stage as observed in the cultivated
390 plots in both years, indicating that substantial crop N uptake must underlie the
increase in MB C:N ratio and/or the decrease in MBN. Hence, soil microbes should have
contributed to crop growth as an N source during later crop growth in all cultivated
plots in this study.

Plant residue treatment clearly affected the relationship between soil microbes
395 (MBN and MB C:N ratio) and crop N uptake during later crop growth in both years: (1)
the period of intense N competition (increased MB C:N ratio) was delayed in the P and
PF plots (Fig. 2c, f); and (2) crop N uptake continued for a longer period in the P and PF
plots than in the C plot (Table 1, Fig. 4, 5). The effects of plant residue application likely
differed between 2007 and 2008 because of the different rainfall patterns between these
400 years. In 2007, there were few heavy rainfall events, although the overall rainfall level
was greater than average. Therefore, N leaching was not excessive, and crop N uptake
and yield in 2007 were greater than average. The soil N pool in the P plot of 2007
increased to 20.0~41.4 kg N ha⁻¹ higher than in the C plot and was similar to the F plot

throughout the experimental period (Fig. 4). However, the increased MBN appeared to
405 suppress crop N uptake temporarily (from 43 to 57 DAP), because crop N uptake in the
P plot (37.0 kg N ha⁻¹; Table 1) was substantially lower than in the C and F plots (50.0
and 65.3 kg N ha⁻¹, respectively; Table 1). Added C substrate and rainfall (77.7 mm in
the same period) in the P plot may have accelerated microbial turnover so that soil
microbes could out-compete the crop for N in the P plot, as previously noted (Bottner et
410 al. 1999; Mayer et al. 2003). As soil dried after 71 DAP (Fig. 1), MBN in the P plot
decreased with increased MB C:N ratio (Fig. 2b, c), and total crop N uptake in the P plot
was similar to that in the C plot at harvest (Table 1), suggesting that the increased
MBN finally contributed to crop growth—i.e., acting as an N source possibly because of
soil drying or crop maturation (Joergensen and Emmerling, 2006; Singh et al., 2007b).

415 By contrast, in 2008 the rainfall was concentrated at the early crop growth
stage, resulting in critical N leaching in all plots (Fig. 1, 5). In this relatively normal
year, the increased MBN consisted of potentially leachable N contributed to
maintaining the larger soil N pool in the P plot compared with the C plot during both
early crop growth (10.5~21.2 kg N ha⁻¹) and later crop growth (~16.3~29.9 kg N ha⁻¹)
420 (Fig. 5). Although the delayed N competition and prolonged crop N uptake period were
similar to 2007 (Fig. 2f, Table 1), increased MBN in the P plot did not seem to limit crop
N uptake as in 2007 (Table 1). Excessive N leaching depressed the soil N pool (especially
inorganic N) in the C plot at 42 DAP (43.2 kg N ha⁻¹), resulting in earlier N competition.

By contrast, increased MBN in the P plot conserved the larger soil N pool at 42 DAP
425 (60.4 kg N ha⁻¹), thus providing much N for crop growth and resulting in the observed
delay in soil N deficiency at 56 DAP, which was the same as the chemical fertilized plots
(F and PF plots) (Fig. 2f, 5). Finally, total crop N uptake in the P plot increased to 19.1
kg N ha⁻¹ higher than in the C plot and was similar to the F plot. On the basis of these
results, soil microbes certainly supplied the potentially leachable N to crop N uptake
430 during later crop growth, although the contribution of mineralized N from applied plant
residue should also be considered. However, the decomposed C in the P plot from 56 to
87 DAP in 2008 was estimated at 240.1 kg C ha⁻¹, so that the amount of net N
mineralization in the P plot during this period was at most 21.2 kg N ha⁻¹ (soil C:N =
11.3), which was less than crop N uptake in this period (31.9 kg N ha⁻¹). Thus, soil
435 microbes immobilizing the potentially leachable N should act as an N source during
later crop growth in the P plot.

5. Conclusions

Our results show that OM application before planting caused immobilization of
440 potentially leachable N by microbes. OM application seemed to improve the soil
microbial N supply and thus foster crop N uptake during the later crop growth,
especially in the year with a critical N leaching. Therefore, plant residue application
before planting would be a promising option to achieve better N synchronization,

although crop yields were relatively higher in the chemical fertilizer plot than in the
445 plant residue plot. Further studies are necessary to evaluate the effect of different
quality and/or quantity of OM application on soil-crop N dynamics, taking into
consideration the rainfall amount and/or distribution (Baijukya et al., 2006; Liu et al.,
2009).

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Figure legends

Fig. 1.

Fluctuation of rainfall (RF) and volumetric water content (VWC) in 2007 and 2008. The
5 experiment was divided into the wet period (–25 to 67 DAP in 2007; –25 to 35 DAP in
2008) and dry period (68 to 103 DAP in 2007; 36 to 103 DAP in 2008) according to
rainfall distribution. The horizontal dotted lines indicate –10 kPa (above) and –1.5 MPa
(below). The downward-pointing arrows indicate each treatment day (P, plant residue
application; F, N fertilizer application; S, seeding; W, weeding; H, Harvest). Asterisk (*)
10 indicates replanting in 2007 because small animals dug out the seeds from the first
planting.

Fig. 2.

15 Temporal variations in microbial biomass C (a, d) and N (b, e), and C:N ratio (c, f) in
2007 (a, b, c) and 2008 (d, e, f). The downward-pointing arrow indicates plant residue
application, and the two upward-pointing arrows indicate N application. Bars indicate
the standard error. Treatments: B, non-cultivated; C, control; F, fertilizer; P, plant
residue; PF, plant residue and fertilizer. Asterisks (*) indicate a significant difference
20 between treatment plots for each sampling time (one-way ANOVA).

Fig. 3.

Temporal variations in CO₂ efflux rate in 2007 (a) and 2008 (b). The downward-pointing
25 arrow indicates plant residue application, and the two upward-pointing arrows indicate
N application. Bars indicate the standard error. Treatments: B, non-cultivated; C,
control; F, fertilizer; P, plant residue; PF, plant residue and fertilizer. Asterisks (*)
indicate a significant difference between treatment plots for each sampling time
(one-way ANOVA).

30

Fig. 4.

Time-course of above-ground crop N uptake and below-ground soil N pool [MBN and inorganic N (NH_4^+ and NO_3^-)] in each plot during the experimental period in 2007. Bars indicate the standard error. Treatments: B, non-cultivated; C, control; F, fertilizer; P, plant residue; PF, plant residue and fertilizer. Different letters (a, b, c, d, and e) indicate that the means of crop N are significantly different ($p < 0.05$) among the sampling times within a plot (one-way ANOVA, Tukey's Kramer test). All values were calculated by multiplying the soil depth (0–15 cm) by the bulk density in each plot ($1.19\text{--}1.27 \text{ g cm}^{-3}$).

40

Fig. 5.

Time-course of above-ground crop N uptake and below-ground soil N pool [MBN and inorganic N (NH_4^+ and NO_3^-)] in each plot during the experimental period in 2008. Bars indicate the standard error. Treatments: B, non-cultivated; C, control; F, fertilizer; P, plant residue; PF, plant residue and fertilizer. Different letters (a, b, c, d, e, and f) indicate that the means of crop N are significantly different ($p < 0.05$) among the sampling times within a plot (one-way ANOVA, Tukey's Kramer test). All values were calculated by multiplying the soil depth (0–15 cm) by the bulk density in each plot (1.17–1.31 g cm^{-3}).

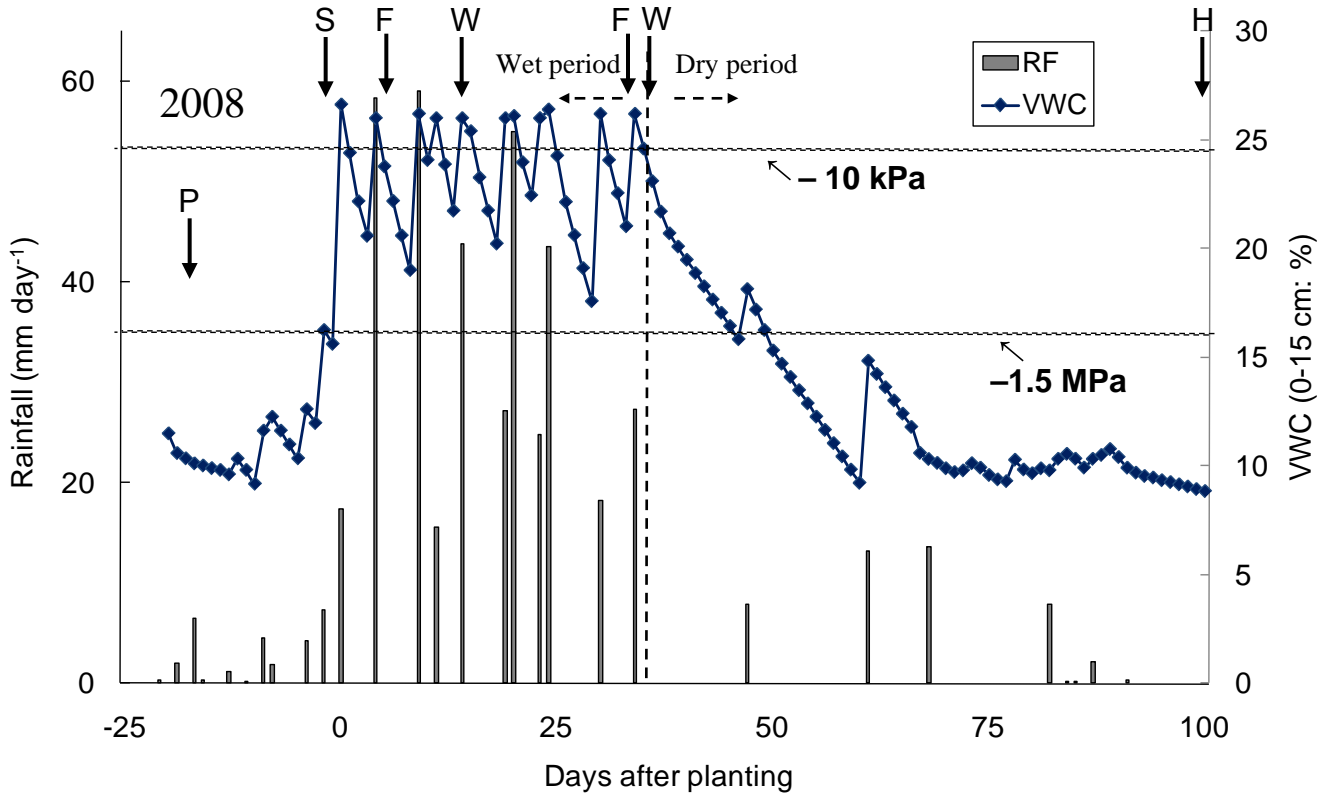
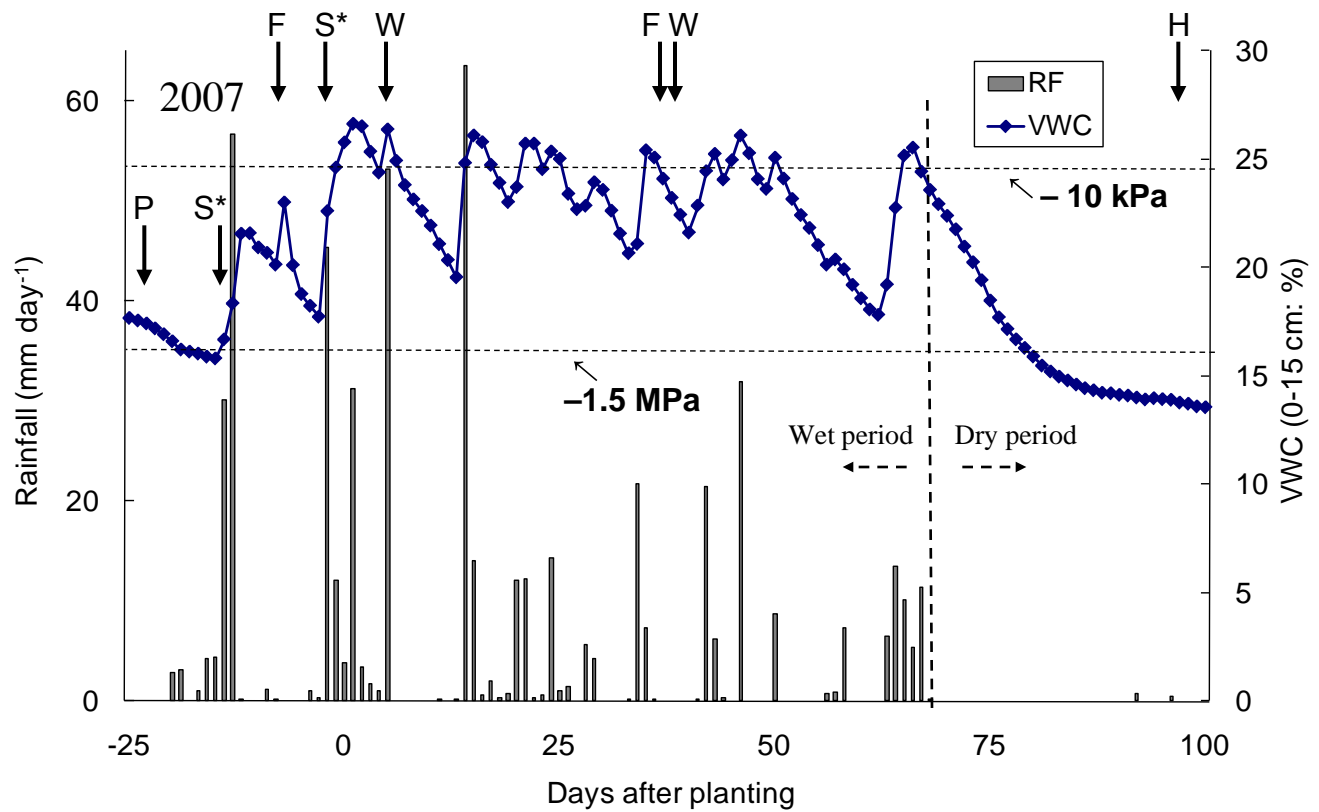
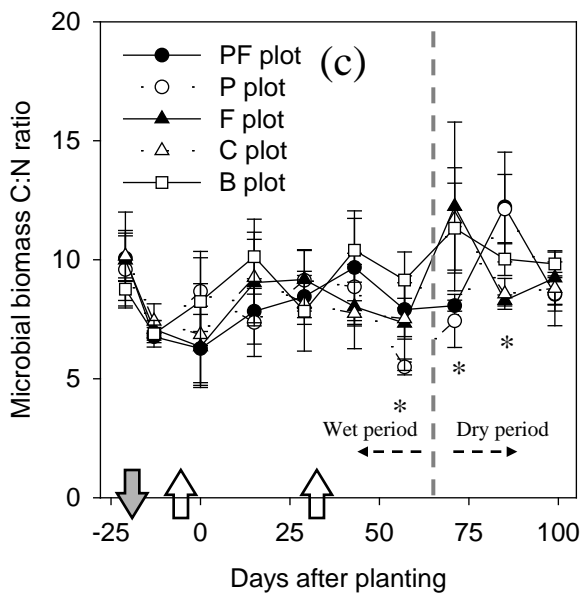
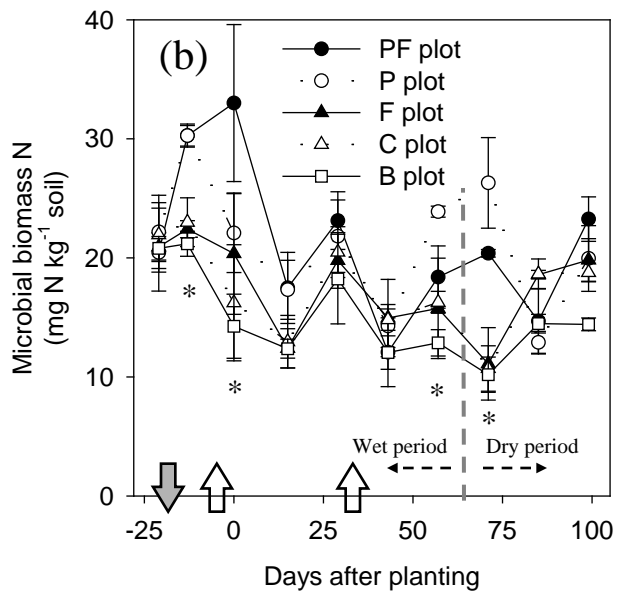
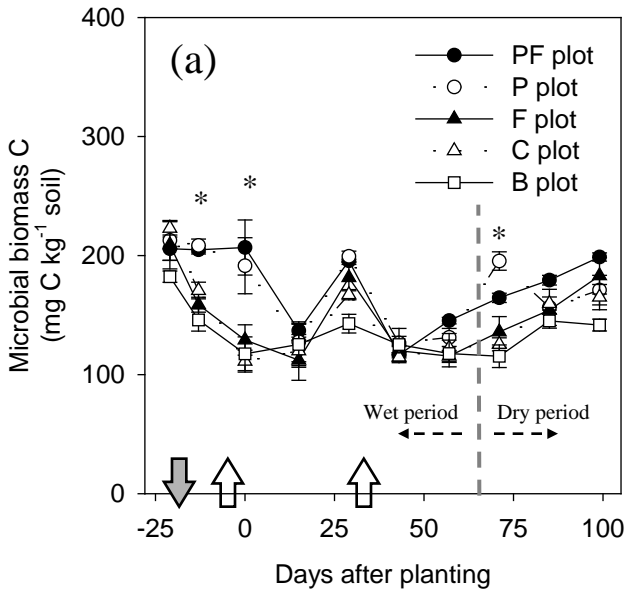


Fig. 1.

2007



2008

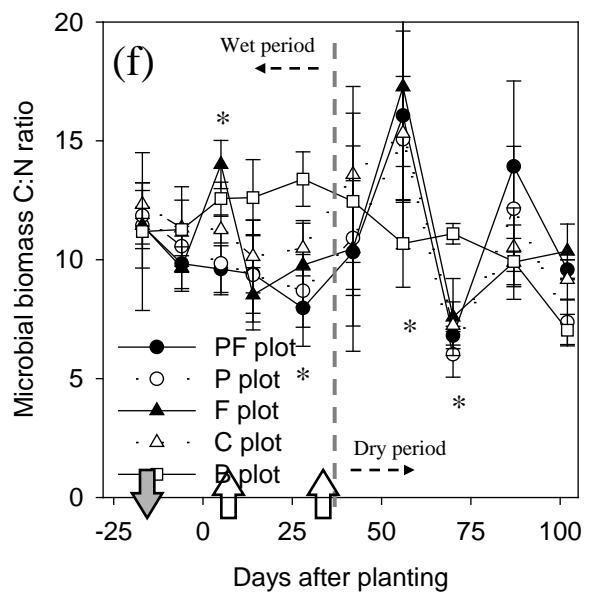
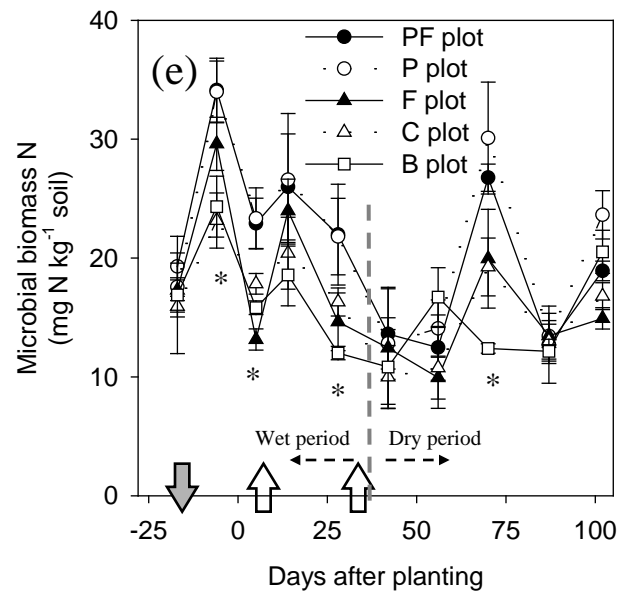
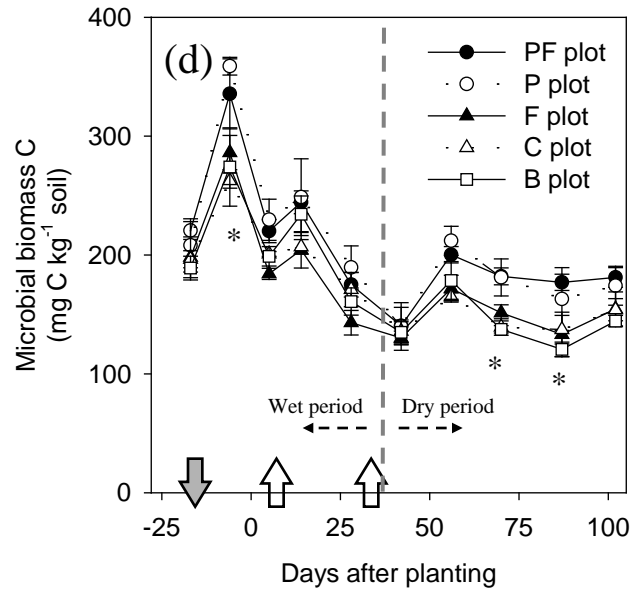
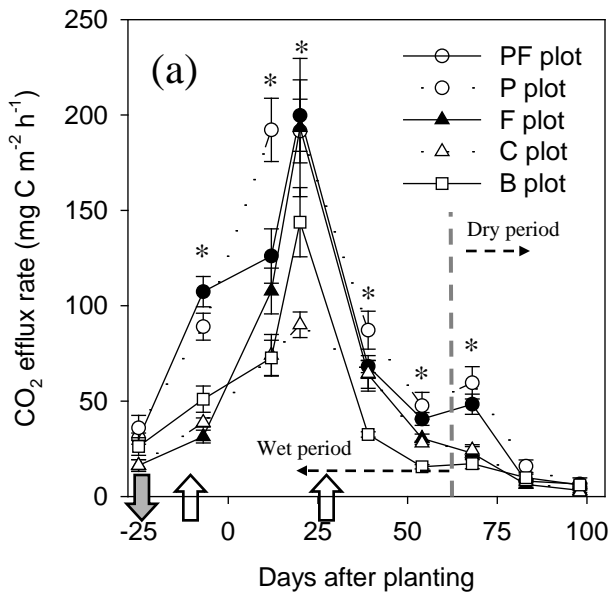


Fig. 2.

2007



2008

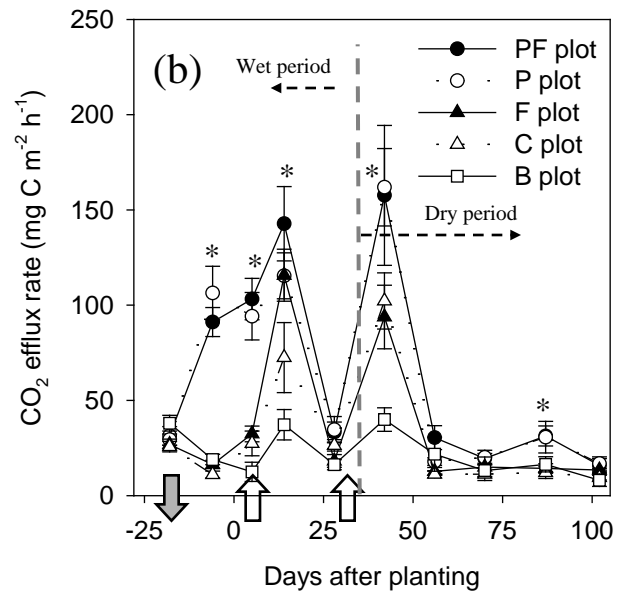


Fig. 3.

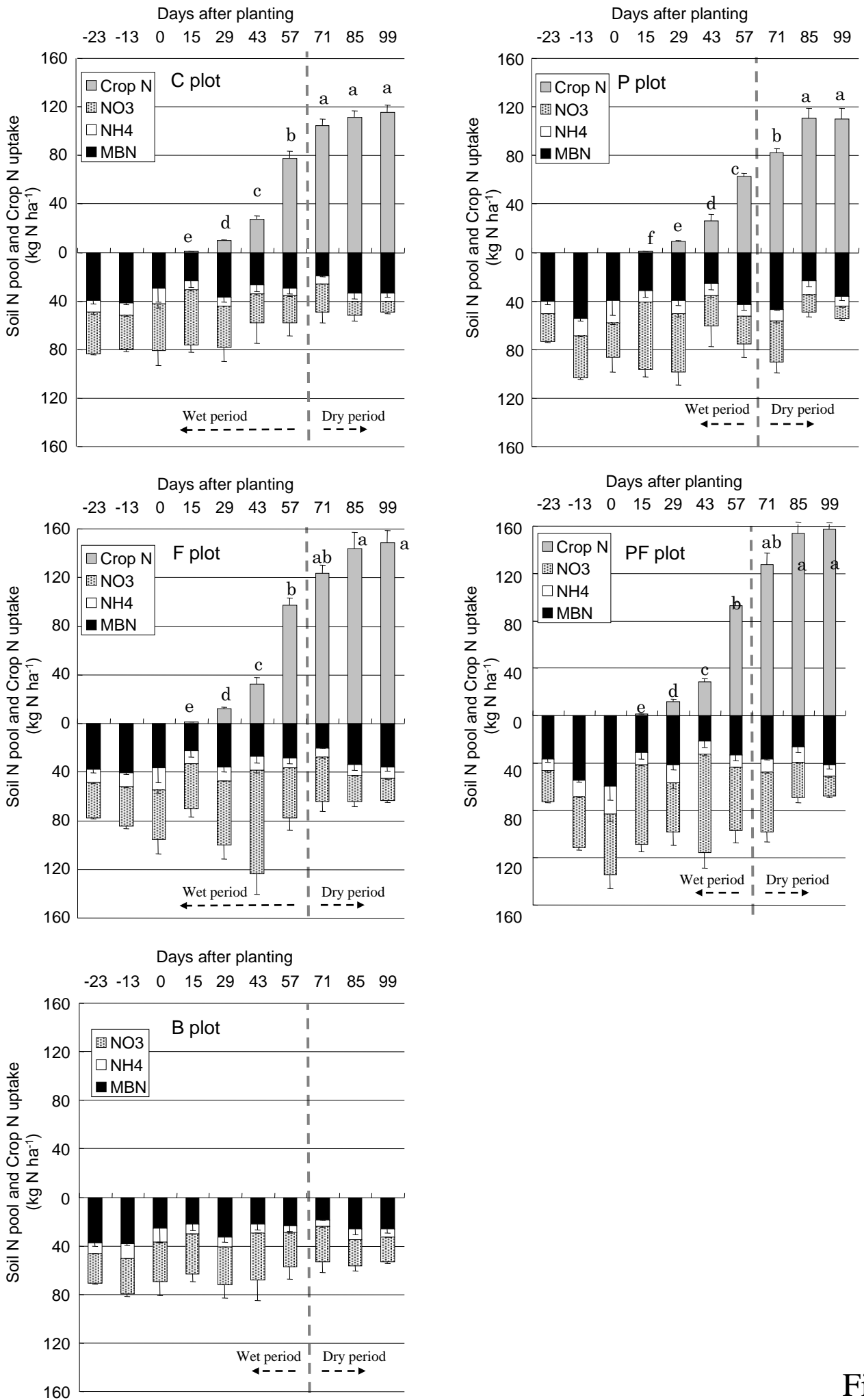


Fig. 4

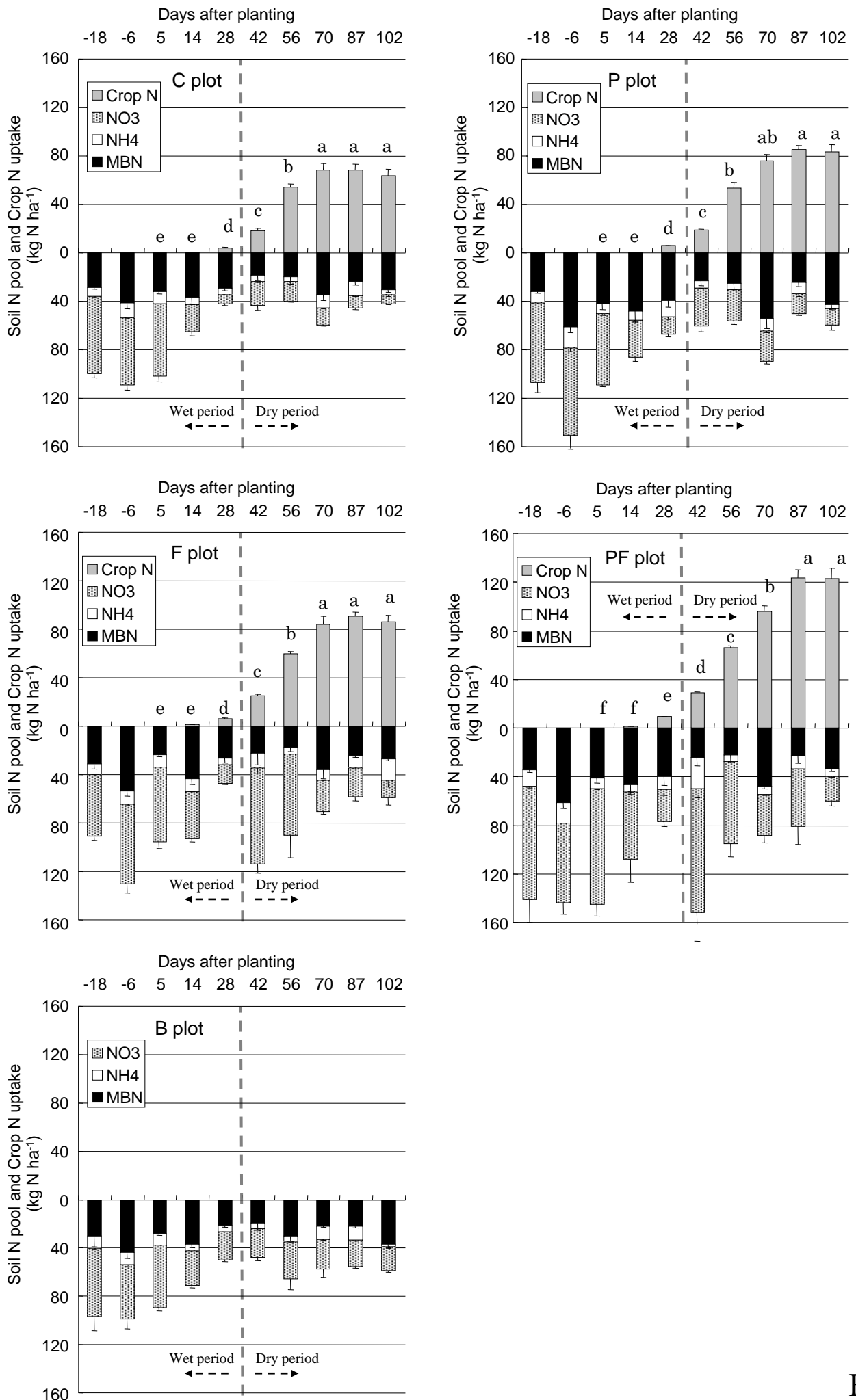


Fig. 5

Table 1. Crop N uptake, and crop yield during the experimental periods in 2007 and 2008.

2007		Crop N uptake (kg N ha ⁻¹)			
		C plot	P plot	F plot	PF plot
N uptake	-25 to -1 DAP	-	-	-	-
	0 to 15 DAP	1.1 (0.1)	0.8 (0.0)	1.6 (0.1)	1.1 (0.1)
	16 to 29 DAP	9.0 (0.6)	8.7 (0.1)	10.7 (1.3)	10.8 (1.7)
	29 to 43 DAP	17.6 (2.7)	16.3 (6.1)	19.9 (5.9)	17.6 (3.7)
	43 to 57 DAP	50.0 (6.3)	37.0 (8.1)	65.3 (9.1)	71.6 (8.4)
	58 to 71 DAP	26.6 (9.0)	23.5 (7.8)	26.4 (11.4)	26.3 (12.2)
	72 to 85 DAP	7.4 (9.8)	23.6 (9.3)	20.2 (15.7)	26.4 (13.6)
	86 to 99 DAP	3.5 (9.9)	0.1 (11.4)	4.4 (16.4)	3.6 (13.0)
	Total	115.1 (7.2) b	109.9 (8.7) b	148.4 (10.0) a	157.3 (10.7) a
Crop yields (t ha ⁻¹)		3.2 (0.2) b	3.2 (0.2) b	4.3 (0.3) a	4.6 (0.2) a
2008		Crop N uptake (kg N ha ⁻¹)			
		C plot	P plot	F plot	PF plot
N uptake	-25 to -1 DAP	-	-	-	-
	0 to 14 DAP	0.6 (0.0)	0.7 (0.1)	1.1 (0.1)	1.0 (0.1)
	15 to 28 DAP	3.7 (0.2)	5.3 (0.2)	5.2 (0.2)	8.4 (0.3)
	29 to 42 DAP	14.0 (2.1)	13.1 (0.7)	19.0 (0.9)	19.5 (1.0)
	43 to 56 DAP	36.0 (6.0)	34.2 (5.3)	34.6 (5.6)	37.1 (5.6)
	57 to 70 DAP	14.4 (8.2)	22.5 (7.9)	24.5 (8.4)	29.6 (10.5)
	71 to 87 DAP	-0.3 (9.1)	9.3 (9.5)	6.2 (10.8)	28.1 (13.0)
	88 to 102 DAP	-4.4 (9.2)	-2.0 (10.5)	-4.2 (11.2)	-1.1 (14.7)
	Total	64.0 (8.9) b	83.1 (9.3) b	86.3 (10.5) b	122.7 (10.7) a
Crop yields (t ha ⁻¹)		1.8 (0.2) b	2.3 (0.2) b	2.7 (0.2) ab	3.6 (0.2) a

Figures in parentheses represent standard error of the means (n=3)

Different letters indicate significant differences between the treatments (p<0.05)

Table 2. Average soil microbial and nutrients variables and CO₂ efflux rate during the experimental periods.

site	treatment	MBC (mg C · N kg ⁻¹ soil)	MBN	MB C/N [#]	NO ₃ ⁻ (mg N kg ⁻¹ soil)	NH ₄ ⁺	CO ₂ efflux rate (mg CO ₂ -C m ⁻² h ⁻¹)
2007	C	147.9 b	17.4 ab	8.6 a	14.9 b	4.6 b	43.7 a
	P	172.0 a	21.1 a	8.4 a	16.5 b	6.2 a	69.5 a
	F	149.9 b	17.6 ab	8.7 a	21.9 ab	6.1 a	59.1 a
	PF	175.5 a	21.3 a	8.6 a	25.7 a	7.1 a	72.0 a
	B	135.9 b	15.1 b	9.3 a	16.0 b	4.6 b	43.6 a
2008	C	177.0 b	16.3 b	11.1 a	15.7 b	4.3 a	30.9 c
	P	210.5 a	21.7 a	10.2 a	19.3 b	5.0 a	62.7 ab
	F	175.7 b	16.9 b	10.9 a	25.3 ab	5.6 a	35.9 bc
	PF	207.7 a	20.9 a	10.5 a	32.5 a	6.1 a	65.7 a
	B	177.3 b	16.2 b	10.8 a	18.1 b	4.1 a	22.2 c

Different letters indicate significant differences between the treatments (p<0.05; n=10)

#; MB C/N = ratio of MBC to MBN

Table 3. F-values according to RM-ANOVA (n=10)

		Crop cultivation (C)	Time (T)	C*T	Plant residue (P)	T	P*T	Fertilizer (F)	T	F*T	P&F	T	P&F*T
MBC	2007	1.48	22.76	2.15	3.96	9.93	2.00	0.10	15.50	0.56	24.40	11.68	1.51
	2008	0.00	44.05	1.02	2.66	46.62	3.30	0.01	76.93	2.16	12.90	49.61	2.35
MBN	2007	1.83	9.48	0.86	11.32	3.52	3.13	0.04	5.27	1.26	8.75	5.56	2.79
	2008	0.02	5.14	1.03	10.07	6.18	0.63	0.27	6.42	0.86	10.88	5.80	0.97
MB C:N	2007	1.12	3.11	2.08	7.90	2.92	1.50	0.34	2.15	1.08	2.05	5.57	1.82
	2008	0.10	2.43	1.60	0.86	2.48	1.08	0.21	2.55	1.17	0.76	2.33	1.70
CO ₂ efflux	2007	0.04	62.51	8.77	16.35	68.29	16.04	4.39	57.71	7.38	23.06	81.85	14.51
	2008	13.29	17.21	5.51	29.48	29.54	5.38	1.89	37.58	1.71	19.85	27.62	4.52

Note: **Bold** character indicates the significance (p<0.05)