



# Microbial biomass, and dissolved organic carbon and nitrogen strongly affect soil respiration in different land uses: A case study at Three Gorges Reservoir Area, South China

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## ABSTRACT

In order to better understand the limiting factors and substrate affecting soil CO<sub>2</sub> flux, we measured total organic carbon (TOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) from seven sites of four land-use types (1 vegetable field, 3 uplands, 2 orchards, 1 pine forest) of subtropical soils in Three Gorges Reservoir Area, China. Objectives were to (1) evaluate the separate and interactive relationship of TOC, MBC, MBN, DOC, and DON with soil CO<sub>2</sub> flux, in addition to the relationship obtained by environmental variables (soil temperature and moisture), and (2) investigate the seasonal and annual CO<sub>2</sub> fluxes from different land uses. Annual CO<sub>2</sub> fluxes ranged from 5.4 to 9.5 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup>. Vegetable field had the highest CO<sub>2</sub> emission, while pine forest had significantly lower CO<sub>2</sub> emission than cultivated land uses. Different quantities of MBC and MBN significantly regulated the CO<sub>2</sub> emission among different land uses, relatively weakly correlated with DOC, while not being correlated with DON. However, temporal fluctuations of CO<sub>2</sub> flux were significantly regulated by MBC, MBN, DOC and DON, in one model of variation, in all land uses. But, when all the variables were included in the multiple stepwise regression analysis, different trend of dominance was observed for soil temperature (two sites), MBC (one site), MBN (one site), DOC (two sites) and DON (one site). Our results indicate that (1) there can be a significant shift of microbial biomass with land-use change, which in turn, caused to shift in CO<sub>2</sub> flux, and (2) apart from the soil temperature, microbial biomass and dissolved organic substances must be considered in a warming future as these can explain a major part of temporal variation of soil CO<sub>2</sub> fluxes.

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## 1. Introduction

Different land uses can have a significant impact on changes of CO<sub>2</sub> fluxes from the soil-to-atmosphere (Iqbal et al., 2008). Land-use changes directed to an emission of 1.7 Pg C per year in the tropics (Reay and Pidwirny, 2006). In China, afforestation area was 0.45 million km<sup>2</sup> in the past 30 years (Houghton, 2002) that included the planting of trees for timber, shelterbelts, fuel wood, and orchards. Such changes may dramatically alter soil organic carbon dynamics (Li et al., 2002) and, in turn, affect exchanges of CO<sub>2</sub> between the soil and the atmosphere (Zhou et al., 2004). Therefore, CO<sub>2</sub> emissions from different land uses are important for our understanding that how environmental variables affect soil C turnover

processes and associated soil CO<sub>2</sub> emissions. Furthermore, forecasts with changes in soil respiration with changes in climate are obviously needed, but they remain highly uncertain in subtropical region.

Factors affecting soil respiration include soil temperature (Hu et al., 2004; Iqbal et al., 2009a), soil moisture (Tang et al., 2006), root exudation (Kuzyakov, 2002) and aboveground plant litter (Raich and Schlesinger, 1992). However, several other soil C and N pools such as soil microbial biomass (SMB) including microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), dissolved organic substances (DOS) including dissolved organic carbon (DOC) and dissolve organic nitrogen (DON) should also be considered while estimating soil respiration budgets. SMB is the most active component of soil organic carbon that regulates biogeochemical processes in terrestrial ecosystems (Paul and Clark, 1996). Although total soil microbial biomass carbon worldwide is approximately 1.4% of the world's total soil organic carbon, its turnover represents a significant contribution to the global carbon cycle (Wardle, 1992). Numerous studies on microbial biomass have been conducted in temperate ecosystems (e.g. Vance and Chapin, 2001). However,

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only a few studies on soil microbial biomass have been performed in subtropical ecosystem. Our understanding of the fluctuation of soil microbial biomass in the vast area of subtropical agriculture soils remains very poor.

Different land uses can have different soil organic carbon (SOC) input rates. These changes in SOC would result in storage/loss of soil C and N, which are likely to influence the pool size and activity of the SMB (MBC and MBN). Due to its highly dynamic character the microbial biomass responds more rapidly than soil organic matter as a whole to changes in management that alter the annual organic input to the soil (Powlson et al., 1987). Since soil microbes are often limited by C and/or N (Wardle, 1992), increased C and N input could provide more resources to support a larger soil respiration processes. Moreover, the microbial community can only access soluble C and consequently it is the concentration, composition and rate of supply of C substrates to the soil solution that drive soil respiration (Van Hees et al., 2005).

Seasonal variation in SMB has been observed in many studies (e.g. Luizão et al., 1998). However, separate seasonal effect and association of MBC and MBN with soil respiration still remain uncertain. Some soil processes can have a strong impact on seasonal variation of SMB, which in turn derives the soil respiration (Lou et al., 2004). Available soil carbon has been commonly recognized as the driving factor regulating soil microbial biomass growth (Wardle, 1992), although other factors such as temperature, soil moisture, soil physical–chemical conditions, and food web interactions may also influence SMB (Coleman and Crossley, 1996). DON represents a significant pool of soluble N in most ecosystems, which have been found to show seasonal variation in temperate agriculture systems (Willett et al., 2004), and can also influence microbial activity. However, its impact on microbial activity (in turn, soil respiration) is still unknown. DOC has also been used as an indicator of carbon availability to soil microorganisms (Boyer and Groffman, 1996). However, observations on the effects of DOC on microbial activity are inconclusive. For example, Burford and Bremner (1975) suggested that DOC was related to heterotrophic microbial processes such as respiration and denitrification, while Cook and Allan (1992) observed that soil respiration rates declined during a 210-day incubation, but DOC content remained constant or increased.

This study explored to what extent the changes in DOS (DOC and DON) and SMB (MBC and MBN) related to seasonal variation of soil CO<sub>2</sub> flux in different land uses in subtropical ecosystem. Different land-use types in the Three Gorges Reservoir Area (TGRA) provided an excellent opportunity to study these issues. The Three Gorges Reservoir Project in China has transformed an entire region, which has remained relatively unchanged for millennia, with unknown environmental consequences (Liu et al., 2004). It is unprecedented anywhere in the world for such significant environmental changes to be made, on such a scale and over such a short period of time. Because of the far-reaching and profound impacts exerted by the building of the Three Gorges Dam (TGD) project due for completion in 2010, even more pressure is now being exerted on this fragile soil resource (Yan et al., 2003). Research on the uncertainty of green house gas (GHG) fluxes in this important area is lacking. The TGD will potentially reduce the coal consumption by 31 million tonnes per year, cutting the emission of 100 million tonnes of greenhouse gases (GGEBC, 2006). However, due to its geographical location and water system, it is of great importance to study the uncertain parameters that affect CO<sub>2</sub> fluxes to provide the necessary references for accurately estimating the CO<sub>2</sub> fluxes at the regional subtropics as well as at global scale.

We hypothesize that soil CO<sub>2</sub> flux will be at the same time significantly influenced by DOS (DOC and DON) and SMB (MBC and MBN) in different land uses as all of these are significantly influenced by climatic season. Furthermore, DOC and DON directly affect plant production and soil microbial activity both of which act as

sources and sinks of DOC and DON in soil. The specific aims of this study were to (1) observe seasonal trend of CO<sub>2</sub> emission during the transition from a dry to wet season in different land-use types; (2) quantify annual CO<sub>2</sub> emissions in dominating agriculture soils in subtropical Southern China; to (3) investigate the seasonal dynamics and differences of DOS (DOC and DON), SMB (MBC and MBN), microbial quotient ( $Q_t$ ), DOC:DON and MBC:MBN ratios between different land-use types, and to establish whether their concentration and dynamics were associated with soil CO<sub>2</sub> flux in different land uses.

## 2. Methods and materials

### 2.1. Site description

The study site was located at the experimental station of Zigui County, TGRA, Southern China (30°38′–31°11′N, 110°18′–111°0′E). Altitude ranges from 133 to 632 m above sea level. The relative humidity is 72%. This region has a typical subtropical monsoon climate with an annual mean air temperature of 18.0°C and an annual frost-free period of 326 days. Annual precipitation averages 1164 mm, of which nearly 80% falls in hot-humid season (April–September) and 20% in the cool-dry season (October–March). The selected sites were representative of the regional land use in TGRA, Southern China. Yellow brown soil of this region derived from granite and/or sandstone, can be referred as Alfisols in the Soil Taxonomy System of China and as Argosols and Cambosols in the Soil Taxonomy System of USA.

Most of the Zigui area is hilly or mountainous with basic farming and green tea (*Camellia sinensis*) production. Arable cultivation on terraced fields is the dominant farming of the county. The average slope of the terrain varies between 5° and 35° and has a southerly aspect. The lower terrain is smooth and the middle–upper terrain is having steep slope. It lies in the upstream of TGRA, and has geomorphological landscape with many mountains after long-time geological evolution and exogenic force erosion of wind and rain. Though dominated by mountains, it is densely populated and has been intensively reclaimed and cultivated. The arable land on steep and dissected terrain is known to be susceptible to soil erosion. Traditional cultivation practices have been sympathetic to soil conservation. Slope length and angles are controlled by dividing slopes into sequences of terraced fields, with associated ditching to divert runoff. Most farmers practice downslope tillage procedures. Although local experimentation has shown that contour cultivation can retain moisture levels as well as reduce erosion hazard (SWCSCC, 1988), downslope tillage is easier to undertake and can assist the drainage of excess water in the earlier part of the monsoon season. Using field mapping combined with a 1:10,000 scale land-use map prepared by the Soil and Water Conservation Bureau of Zigui County, the proportion of land cover types in the catchments is as follows: Upland 28.8%; Paddy 29.1%; Orchard 12.3%; Woodland 20%.

The experimental sites were located within Zigui County, including two watersheds; Zhangjiachong (1.62 km<sup>2</sup>) about 12 km southwest of Three Gorges dam, Quxi (17.6 km<sup>2</sup>) about 4.5 km northwest of Three gorges dam. These watersheds are about 40 km away from each other. The sites were selected to cover the major land uses in this area. From Zhangjiachong, four sites were chosen to represent different land uses: Z-1—upland, Z-2—upland, Z-3—orchard, and Z-4—pine forest. In 2003, Z-1 was transferred from paddy field to arable land having corn, peanut and sweet potatoes, while *C. sinensis* seedlings were inter-planted in autumn 2006. Z-2 was a wasteland before 1922 when it was planted with *C. sinensis* seedlings which lasted up to 1967. In 1967, Z-2 land usage was replaced with Chinese chestnut (*Castanea mollissima*) which started

**Table 1**  
Description of land use, selected physico-chemical properties of the soils and fertilizer application rate in different land-use types at the Zigui County.

Site code	Land use	Slope	pH	SOC (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	BD (g cm <sup>-3</sup> )	Particle size distribution (%)			Tillage practice	Source of fertilizer	Amount of N fertilizer applied (kg ha <sup>-1</sup> year <sup>-1</sup> )
							Sand	Silt	Clay			
Z-1	Upland transferred from paddy	Terrace	6.51 ± 0.02b	25.1 ± 0.12b	1.08 ± 0.01	1.29 ± 0.01b	54.4	30.5	15.0	No	Ammonium bicarbonate + urea	732
Z-2	Upland	Terrace	6.42 ± 0.03bc	20.8 ± 0.19d	1.02 ± 0.01a	1.50 ± 0.02a	77.0	16.0	7.0	No	Urea	286
Z-3	Orchard	Terrace	6.17 ± 0.02de	17.8 ± 0.10e	1.10 ± 0.02a	1.49 ± 0.02a	83.0	12.5	4.5	No	Urea	709
Z-4	Woodland	>25°	6.75 ± 0.03a	23.9 ± 0.27c	1.13 ± 0.02a	1.35 ± 0.01b	64.5	22.5	13.0	No	–	–
Q-1	Vegetable field	Terrace	6.54 ± 0.01e	20.1 ± 0.13d	1.12 ± 0.01a	1.34 ± 0.02b	54.5	28.0	17.5	No	Manure + urea	557
Q-2	Upland	Terrace	6.21 ± 0.02de	29.1 ± 0.22a	1.05 ± 0.01a	1.28 ± 0.01b	54.5	25.5	20.0	No	Ammonium bicarbonate + urea	507
Q-3	Orchard	Terrace	6.32 ± 0.01cd	23.8 ± 0.14c	1.06 ± 0.02a	1.54 ± 0.02a	67.0	22.0	11.0	Twice a year	Urea	699

Means in a column followed by the same letter were not significantly different at  $P \leq 0.05$ , by Duncan's multiple range tests. Values are the mean ± S.E. (standard error). Q, Quxi watershed; Z, Zhangjiachong watershed; SOC, soil organic carbon; BD, bulk density.

to have a rotation with rapeseed in 1983, and is maintained until now. From 1967 to 1983, Z-3 was cultivated with *C. mollissima*. In 1983, it was replaced by orange trees. In 1980, Z-4 was vegetated with Chinese red pine (*Pinus tabulaeformis*). Before their respective vegetation, Z-3 and Z-4 were wastelands with low soil quality resulting from intensive soil erosion. Until now, these are well being used according to their above described land-use patterns. Distance among Z-2, Z-3 and Z-4 is about 10 m from each other and these sites are about 200 m away from Z-1. From Quxi, three sites were chosen to represent different land uses; Q-1—vegetable field, Q-2—upland, and Q-3—orchard. For Q-1, land-use change occurred to produce various vegetables (Chili (*Capsicum annuum*), Chinese cabbage (*Brassica rapa*), tomato (*Lycopersicon esculentum*), coriander (*Coriandrum sativum*)) inter-planted with soybean and canola in 2003, before which, it was a paddy field. For Q-2, land-use change occurred to have rapeseed–sweet potato rotation in 1978, before which, it had *C. sinensis* plantation. While for Q-3, land-use pattern was changed to have orange trees in 1997, before which, it had *C. sinensis* plantation. Until now, these three sites are properly managed to produce the above described pattern of their respective land usage. Distance among Q-1, Q-2 and Q-3 is about 10 m from each other. Organic and/or inorganic fertilizers were used for all the land-use types except Z-4. Owing to high soil test levels, no P and K fertilizer was applied in all land uses. The description of fertilizer applied, and the relevant chemical and physical properties of the sites are listed in Table 1.

## 2.2. Experimental setup

In January 2008, an experiment from seven sites was established to measure CO<sub>2</sub> fluxes. In each field, three chambers were permanently installed (each 5 m apart) in a triangle form, and the monthly measurements were made over the whole experiment (20 January 2008 to 20 December 2008). During the measurements, the soil surface within the chamber was kept free of any live vegetation by clipping the seedlings off with a scissor. All the field measurements were made in the morning at 09:00–11:00. The 2 h data were used for two consecutive measurements average. The monthly measurements were supposed to only represent temporal variation of CO<sub>2</sub> fluxes and other environmental variables. However, these may not represent discrete fertilized and rainfall events.

## 2.3. CO<sub>2</sub> flux measurements

Soil CO<sub>2</sub> fluxes were measured by using static close chambers with a portable infrared gas analyzer ZEP-5 (ZEP-5, Fuji Electric Co., Ltd., Tokyo, Japan). The closed chamber was made from 8 mm thick acrylic material with a diameter and height of 20 and 25 cm, respectively. The top round edges were rubber-sealed in order to prevent from leakage when the top lid was put on it. The round box was inserted directly 5 cm into the soil, and the cover was placed on top during sampling and removed afterwards. The chambers were equipped with 1 m vents (diameter 1 mm) for pressure equilibration. The chambers were insulated to reduce the impact of direct radiative heating during the gas sampling.

Samples were taken with 50 ml plastic syringes attached to a three-way stopcock at 0, 10, 20, 30 min following chamber closure, respectively, and then injected into evacuated bags made of inert aluminum-coated plastic. The CO<sub>2</sub> concentrations in the bags were analyzed using the portable infrared gas analyzer within 24 h following sampling. The infrared gas analyzer configurations and calculation of the gas flux were the same as described by Hu et al. (2004). The soil CO<sub>2</sub> emissions (expressed as mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) were

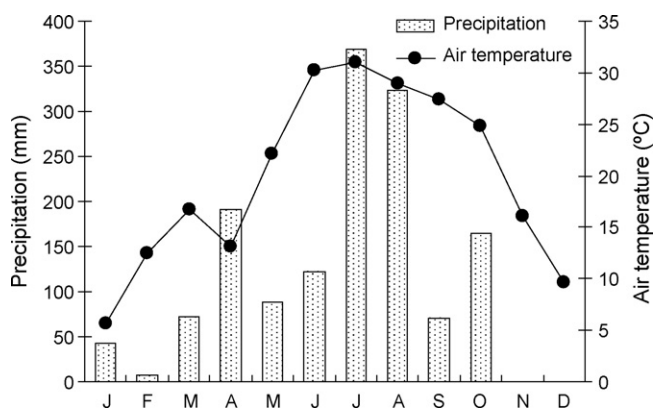


Fig. 1. Monthly variations of precipitation and air temperature at Zigui County.

calculated as follows:

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{T} \times \alpha$$

where  $F$  ( $\text{mg C m}^{-2} \text{ h}^{-1}$ ) is the soil respiration rate,  $\rho$  is the density of  $\text{CO}_2$  ( $1.98 \times 10^3 \text{ g m}^{-3}$ ) under standard condition,  $V$  ( $\text{m}^3$ ) and  $A$  ( $\text{m}^2$ ) are the volume and bottom area of the chamber, respectively,  $\Delta c$  ( $\text{m}^3 \text{ m}^{-3}$ ) is the change in  $\text{CO}_2$  concentration in the chamber during the period  $\Delta t$  (h),  $T$  is the absolute temperature and  $\alpha$  is the conversion factor for  $\text{CO}_2$  to C (12/44). The annual total soil  $\text{CO}_2$  fluxes were calculated by multiplying the rate (average of three replications) by 28–31 days (the interval time). It should also be noted that data measured in the closed chamber in this study may possibly be underestimated by around 10% (Rayment, 2000).

#### 2.4. Soil sampling and analysis

During 1 year, monthly soil samples (0–20 cm) were collected from each field, at the same time of  $\text{CO}_2$  flux measurements, mixed and placed in plastic bags after manual removal of visible plant residues and roots, immediately stored in an ice chest until they were transported to the laboratory. From each site, five subsamples (0–20 cm depth) were collected (with a probe of 6.0 cm diameter core) at random and composited into one soil sample for each of the three replicates. Field-moist soil samples were divided into two subsamples. One subsample was used for TOC, MBC, MBN, DOC, and DON measurements. Another was allowed to dry at room temperature, and used for chemical analyses.

Air-dried soil samples were sieved, and analyzed for total N (Kjeldahl method), and pH (1:1 soil water paste) with electrometry (pH electrode). Soil bulk density was determined by retrieving undisturbed cores of known volume from each sampling site subsequently being oven-dried at  $105^\circ\text{C}$  until constant weight was achieved. Particle size distribution was analyzed with pipette method using pyrophosphate as dispersing agent.

For TOC, MBC, MBN, DOC and DON determinations, soil samples were stored at  $5\text{--}7^\circ\text{C}$  pending analyses. Prior to analysis soil samples were sieved through a 2-mm sieve, adjusted to a 40% of water holding capacity, and kept at room temperature for 7 days. MBC and MBN were estimated by the chloroform fumigation-extraction method (Brookes et al., 1985; Wu et al., 1990), using alcohol free  $\text{CHCl}_3$ , followed by 0.5 M  $\text{K}_2\text{SO}_4$  extraction of both fumigated and non-fumigated soils. Both the non-fumigated and fumigated soil extracts were filtered through Whatman # 40 paper and frozen until analysis of TOC, MBC, MBN, DOC and DON. Soil MBC was analyzed by a TOC-5000A total organic C analyzer (Shimadzu, Kyoto, Japan), and estimated using the equation:  $\text{MBC} = 2.22\text{Ec}$ , where Ec is the difference between organic C extracted from the  $\text{K}_2\text{SO}_4$  extracts

of fumigated and non-fumigated soils, both expressed as  $\mu\text{g C g}^{-1}$  oven dry soil (Wu et al., 1990). The same  $\text{K}_2\text{SO}_4$  soil extract was used to determine MBN. The extractable total nitrogen from fumigated and non-fumigated soil was measured by the total persulfate oxidation procedure (Keeney and Nelson, 1982). MBN was calculated as the difference between the extractable total nitrogen of fumigated and non-fumigated soil, using the following equation (Solaiman, 2007):  $\text{MBN} = E_N / 0.45$ , where  $E_N$  is the difference between the amounts of total N extracted from the  $\text{K}_2\text{SO}_4$  extract of fumigated and non-fumigated soil both expressed as  $\mu\text{g N g}^{-1}$  oven dry soil, and 0.45 is the fraction of biomass N extracted after chloroform fumigation.

For convenience, it is preferable to analyze DOC and DON in routine extractions undertaken either for inorganic N or microbial biomass determination in soil (Horwath and Paul, 1994; Mulvaney, 1996). Therefore, DOC was analyzed from the same extract taken from non-fumigated soils (Hogberg and Hogberg, 2002), and measured by dichromate oxidation method (Jenkinson and Powlson, 1976). DON was calculated as the difference between the total dissolved nitrogen (TDN) reading and the combined  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (dissolved inorganic nitrogen, DIN) reading (Jones and Willett, 2006). From non-fumigated soil extract, TDN was determined by persulfate oxidation method (Hagedorn and Schleppi, 2000). Soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were measured from non-fumigated soil extracts using cadmium reduction followed by a modified Griess–Ilosvay method and indophenol blue reaction (Mulvaney, 1996).

#### 2.5. Measurement of environmental factors

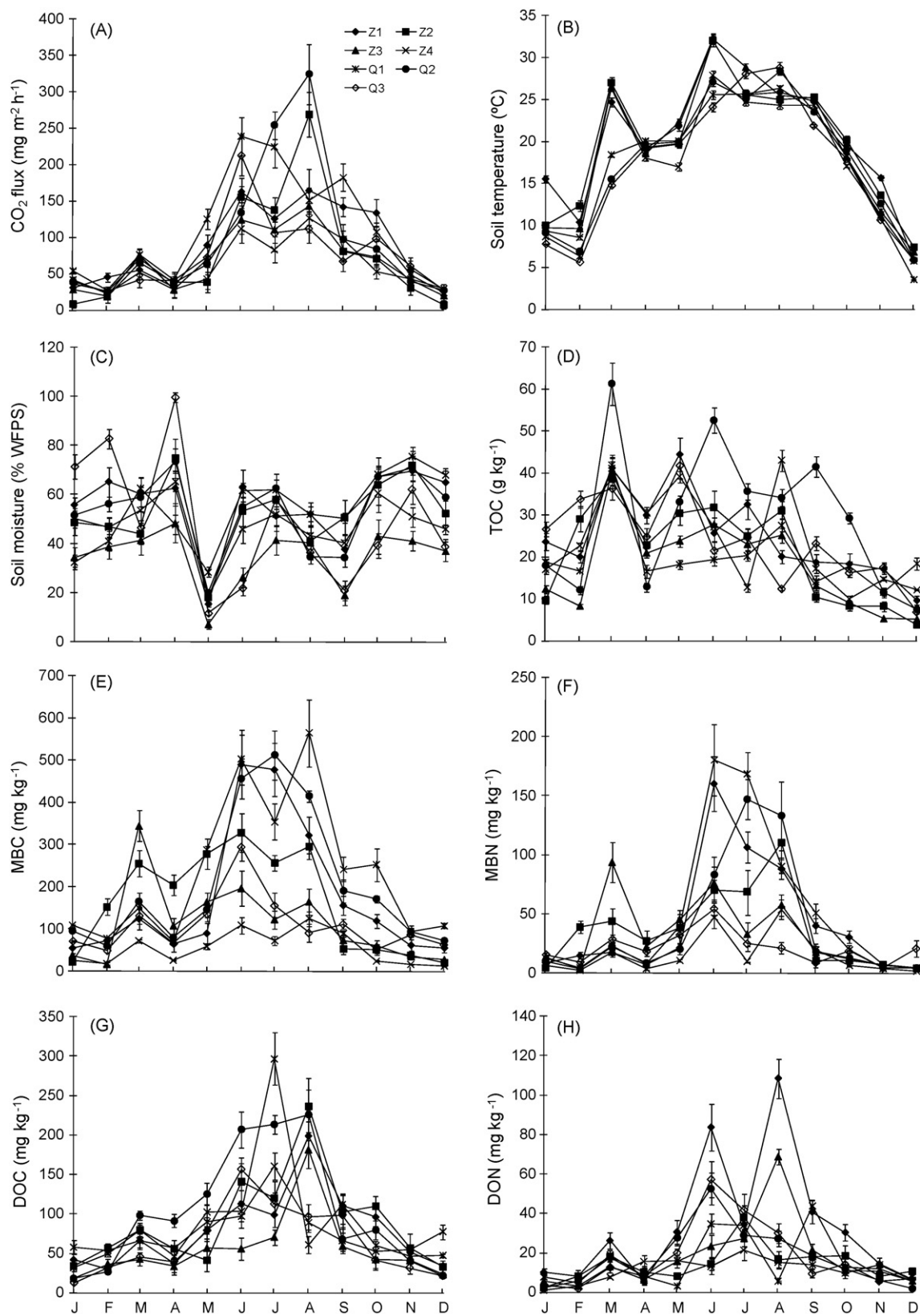
Soil moisture content at a depth of 10 cm, was estimated by the relative water content as the percentage of water-filled pore space (WFPS). The soil water content ( $\text{g g}^{-1}$ ) was determined by gravimetry with oven drying at  $105^\circ\text{C}$  for 24 h. WFPS was calculated with the following equation (Franzluebbers, 1999):  $\text{WFPS} = (\text{SWC} \times \text{BD}) / [1 - (\text{BD}/\text{PD})]$ , where SWC is the soil water content ( $\text{g g}^{-1}$ ), BD is the soil bulk density ( $\text{g cm}^{-3}$ ), and PD is the soil particle density. Soil temperatures were measured using soil thermometers inserted to a depth of 5 cm inside the chambers. Climatic data (precipitation and air temperature) were obtained at the weather station of soil and water conservation station of Zigui County.

#### 2.6. Statistical analysis

Most of the statistical analyses were performed with SAS software (version 8; SAS Institute, Cary, NC). When necessary, data were log-transformed to meet the assumption of normal distribution.  $\text{CO}_2$  fluxes, soil temperature, WFPS, MBC, MBN, DOC, DON and TOC for each land use were calculated by averaging the 3 replicates on each sampling day. A repeated-measure analysis of variance was used to test the differences of environmental factors, MBC, MBN, DOC, DON and  $\text{CO}_2$  fluxes among the seven land uses, season and to assess the significance of the impacts of land use, season, and their interactions on  $\text{CO}_2$  fluxes. The soil temperature effect on soil respiration was linearized with a  $Q_{10}$  function, which assumed an optimum respiration at  $30^\circ\text{C}$  and doubled with every  $10^\circ\text{C}$  change in temperature (Kucera and Kirkham, 1971):

$$\text{Temperature function} = 2^{[(\text{C}-30)/10]}$$

Soil  $\text{CO}_2$  fluxes were linearly regressed with soil temperature, WFPS, MBC, MBN, DOC and DON, separately as well as possible interaction terms, for each land-use type. To see the dominant variable regulating soil  $\text{CO}_2$  fluxes, step wise regression analysis between  $\text{CO}_2$  fluxes and different environmental variables was per-



**Fig. 2.** Seasonal variation of (A) soil CO<sub>2</sub> flux, (B) soil temperature at 5 cm depth, (C) soil moisture (% WFPS, 0–10 cm), (D) total organic carbon (TOC), (E) microbial biomass carbon (MBC), (F) microbial biomass nitrogen (MBN), (G) dissolved organic carbon (DOC) and (H) dissolved organic nitrogen (DON). WFPS indicates water-filled pore space. Values are mean ( $\pm$  standard error) determined in the morning (09:00–11:00 a.m.) on sampling dates.

**Table 2**Significance of the impacts of land use, season, and their interactions on soil temperature, soil moisture, MBC, MBN, DOC, DON, TOC, and CO<sub>2</sub> at the Zigui County.

Property	Soil temperature	Soil moisture	MBC	MBN	DOC	DON	TOC	CO <sub>2</sub>
Land use	ns	ns	**	**	**	*	*	**
Season	**	**	**	**	**	**	ns	**
Land use vs season	ns	ns	ns	ns	ns	ns	ns	ns

ns, no significant impact.

\* Significance impact at  $P \leq 0.05$ .\*\* Significance impact at  $P \leq 0.001$ .

formed by using SPSS 12.0 software package. A  $P$ -value  $<0.05$  was used to reject the null hypothesis that the model is not significant.

### 3. Results

#### 3.1. Environmental factors

Over the 1-year-study period, the annual rainfall (January–December) was significantly higher (1449.6 mm) than the long-term average annual rainfall of 1164 mm. Intense rainstorms occurred in April, July and August. Precipitation during the period of April to September accounted for 80.3% of total rainfall in the year. The test sites frequently experiences heat stress during growing season, with the maximum daily temperature in excess of 30 °C. Annual mean air temperature was 19.9 °C, with monthly temperature ranging from 5.7 °C (January) to 31.0 °C (July) (Fig. 1).

A distinct seasonal difference of soil temperature can be observed (Table 2 and Fig. 2B). However, no significant differences in soil temperature were observed among the seven land-use types (Table 2). In this study, although higher rainfall occurred during April–September and lower rainfall occurred during October–March, higher WFPS was found during October–March than April–September (Figs. 1 and 2C). We divided the 1-year period into two seasons according to the rainfall: (1) April through September (the hot-humid season) and (2) from October through March (the cool-dry season). Although, WFPS also showed significant seasonal response, soil temperature was not found to be well correlated with WFPS across the season (data not shown). The seasonality of soil temperature coincided with the seasonal patterns of air temperature (Figs. 1 and 2B).

#### 3.2. Microbial biomass C and N, and dissolved organic C and N

Soil microbial biomass C showed clear seasonal variation during the study period. Soil MBC varied among months within the range of 13.1–564.3 mg C kg<sup>-1</sup> (Fig. 2E). MBC was higher in hot-humid season than in cold-dry season (Fig. 2E). MBC peaked in June, July and August when there were maximum air temperature with some intense rainstorms. While the lowest level occurred in December when there was no rainfall. During the whole study period, MBC significantly differed among different land uses following the order of Q-1 > Q-2 > Z-1 > Z-2 > Z-3 ~ Q-3 > Z-4 (Fig. 2E). No significant land use–season interaction was observed, as the same seasonal trend was observed from all land uses (Table 2). Soil MBN almost showed similar trend of variation as observed for MBC. MBN from different land uses varied from 1.4 to 180.3 mg N kg<sup>-1</sup>, and was significantly different following the order of Q-1 > Z-1 > Q-2 > Z-2 > Z-3 > Q-3 > Z-4 (Fig. 2F).

Soil MBC quotient ( $Q_t$ ) was calculated as fractions of soil TOC. The magnitude of  $Q_t$  or the proportions of microbial biomass C in soil TOC was significantly higher in cultivated soils than in pine forest (Z-4) suggesting that forest litter can be less suitable as microbial substrate compared with cultivated litter. The  $Q_t$  value of non-tilled orchard (Z-3) was significantly higher than tilled orchard

(Q-3), which indicates that tillage caused to disturb the microbial activities in the soil. Among the cultivated sites, the  $Q_t$  value was significantly different following the order of Q-1 > Z-1 ~ Q-2 > Z-2 > Z-3 > Q-3.

The seasonal pattern of DOC and DON was similar to MBC and MBN, with some peaks being observed during the months of intense rainstorms, except that DON levels decreased significantly in July when intense rainstorms with higher magnitude occurred. However, unlike MBC and MBN, DOC and DON had different orders of magnitude in different land uses. From all land uses, the DOC concentration ranged from 12.4 to 297 mg C kg<sup>-1</sup> and was significant in the following order: Q-2 > Z-2 ~ Q-1 > Z-1 ~ Z-4 > Q-3 > Z-3 (Fig. 2G). Unlike DOC, DON did not show highly significant amount of difference among different land uses, and was slightly significant in the following order: Z1 > Z3 ~ Q3 ~ Q2 > Q1 ~ Z2 > Z4 (Fig. 2H). Neither the DOC concentration nor the DON pool had significant land use × season interaction (Table 2).

#### 3.3. Seasonal variations in CO<sub>2</sub> fluxes

Emission of CO<sub>2</sub> is likely influenced by precipitation directly and indirectly. Highest precipitation was observed in hot-humid season when CO<sub>2</sub> emission emerged with some peaks. Soil CO<sub>2</sub> emission positively correlated with precipitation in all land-use types except Q-3 (data not shown). In all sites, soil CO<sub>2</sub> fluxes were significantly higher in the hot-humid season (April–September) than in the cool-dry season (October–March) ( $P < 0.001$ , Fig. 2A). Over the whole experimental period, maximum CO<sub>2</sub> release (324.44 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) took place in August when soil temperature was relatively high (28.4 °C) and moisture was relatively low (WFPS of 35%) (Fig. 2A, B and C). Minimum emissions (7.2 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) occurred in December when soil temperature was low (7.4 °C), and soil moisture was at moderate level (WFPS of 52%) (Fig. 2A, B and C). The CV values of CO<sub>2</sub> fluxes in dry season (29.5–85.2%) were more variable than those in humid season (43.1–74.8%), which indicated a higher temporal variability in dry season than in humid season.

#### 3.4. Land-use effect on CO<sub>2</sub> fluxes

Land use had a significant impact on soil CO<sub>2</sub> efflux (Table 2). Soil CO<sub>2</sub> fluxes significantly differed among the soils under different land uses, following the order of Q-1 > Q-2 > Z-1 > Z-2 ~ Q-3 > Z-3 > Z-4 (Fig. 2A). A significantly higher CO<sub>2</sub> flux was found from vegetable field, uplands, and orchards as compared to pine forest (Z-4), as higher amounts of MBC and MBN were found from vegetable field, uplands, and orchards than pine forest, indicating that either more N or C is available in cultivated soils for immobilization or there are certain conditions in cultivated soil substrates which cause immobilization of C and N. Soil CO<sub>2</sub> flux was higher in Q-3 (orchard with tillage) than in Z-3 (orchard without tillage) (Fig. 2A), which indicated a significant flux difference induced by tillage practice. The annual site CO<sub>2</sub> emission rates, calculated by integration of the mean emission on each sampling occasion, were estimated as  $9.5 \pm 0.47$ ,  $8.8 \pm 0.53$ ,  $7.8 \pm 0.46$ ,  $6.8 \pm 0.37$ ,  $6.6 \pm 0.26$ ,

**Table 3**  
Soil CO<sub>2</sub> flux (mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) as predicted by soil temperature (*T*) (i.e., linearized transformation from  $2^{[(T-C-30)/10]}$ ), water-filled pore space (*W*), microbial biomass carbon (MBC, mg kg<sup>-1</sup>), microbial biomass nitrogen (MBN, mg kg<sup>-1</sup>), dissolved organic carbon (DOC, mg kg<sup>-1</sup>), and dissolved organic nitrogen (DON, mg kg<sup>-1</sup>) from seven land-use types at the Zigui County.

Property	Site code																				
	Z-1			Z-2			Z-3			Z-4			Q-1			Q-2			Q-3		
	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t	‡	R <sup>2</sup>	P> t
Sources of variation of soil CO <sub>2</sub> flux																					
Intercept	−230.2		***	−119.9		**	−18.86		***	−547.4		**	−140.8		***	−31.60		***	−24.38		*
T	350.1	0.61	**	291.7	0.47	*	62.31	0.74	***	1183.7	0.62	**	210.2	0.78	***	351.9	0.69	***	89.72	0.45	*
W	−0.39	0.06	ns	−0.11	0.02	ns	0.35	0.03	ns	0.10	0.03	ns	0.003	0.007	ns	0.69	0.07	ns	0.71	0.25	ns
MBC	4.19	0.53	**	1.44	0.39	*	0.44	0.30	ns	1.12	0.86	***	0.63	0.78	***	2.27	0.77	***	2.88	0.68	***
MBN	3.92	0.53	**	3.08	0.78	***	1.94	0.44	*	1.31	0.80	***	2.41	0.77	***	2.57	0.91	***	3.60	0.53	**
DOC	3.39	0.79	***	2.88	0.95	***	0.89	0.60	**	1.15	0.23	ns	2.24	0.50	**	1.31	0.75	***	0.19	0.75	***
DON	2.93	0.73	***	−2.42	0.26	ns	3.07	0.71	***	−0.11	0.23	ns	−2.36	0.58	**	−7.48	0.34	*	3.73	0.85	***
T × W	−4.64	0.36	*	−5.88	0.32	ns	−0.13	0.51	**	−22.41	0.38	*	−5.04	0.72	***	−4.63	0.36	*	−0.03	0.01	ns
T × W × MBC × MBN	−0.08	0.35	*	0.07	0.80	***	−0.001	0.18	ns	0.27	0.78	***	0.07	0.66	***	0.05	0.84	***	0.03	0.70	***
T × W × DOC × DON	0.16	0.55	**	0.04	0.73	***	0.01	0.46	**	0.002	0.22	ns	0.02	0.56	**	0.04	0.46	**	0.13	0.87	***
T × W × MBC × MBN × DOC × DON	0.02	0.40	*	0.21	0.87	***	0.03	0.46	**	1.03	0.80	***	0.58	0.59	**	0.13	0.70	***	0.001	0.74	***
Summary statistics																					
R <sup>2</sup>		0.89			0.98			0.96			0.93			0.89			0.94			0.92	
RMSE		25.9			13.5			12.1			13.3			36.7			35.8			22.5	
P>F		0.002			<0.001			0.002			0.008			0.003			<0.001			<0.001	
COV		29.2			17.6			17.9			21.6			33.9			35.7			23.0	

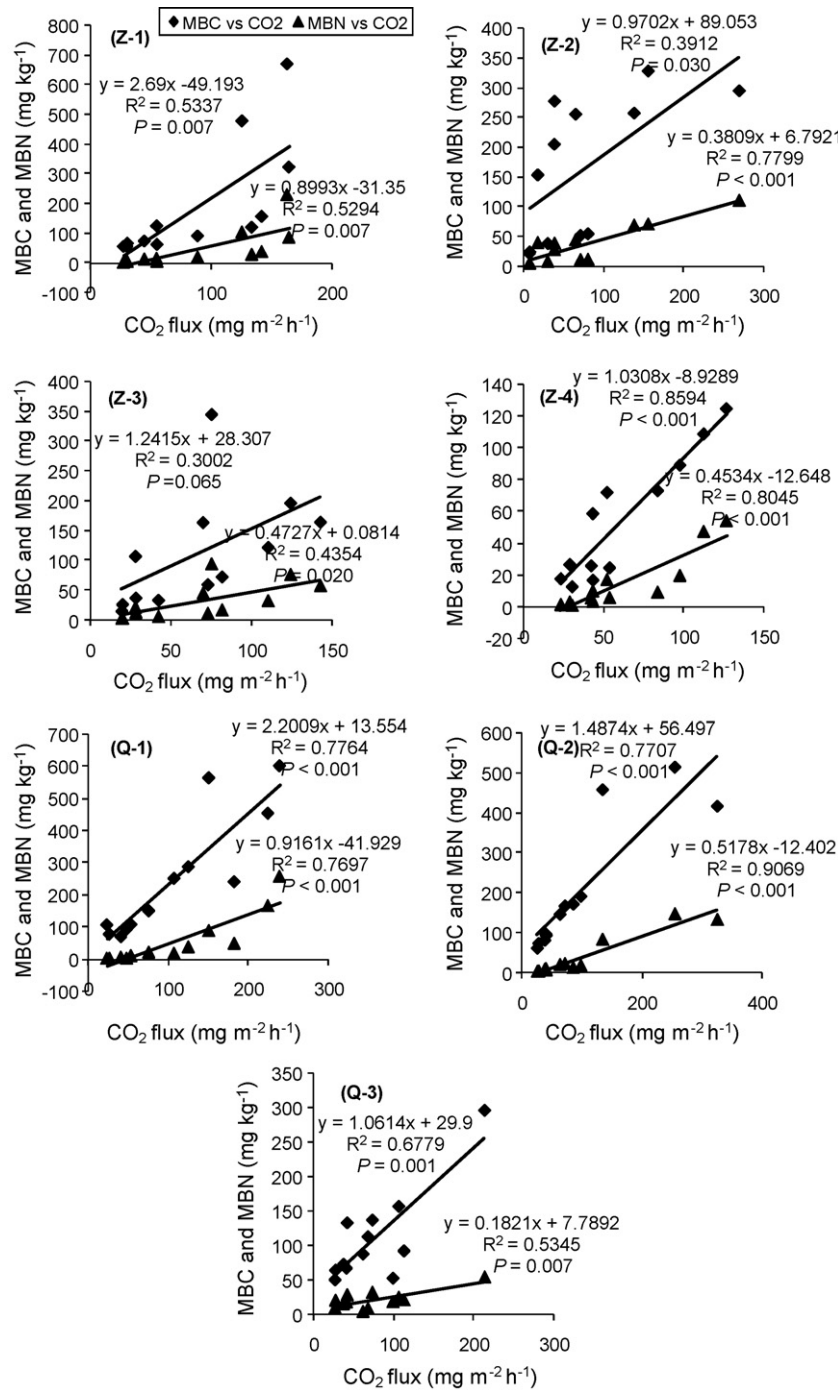
RMSE, root mean square error; COV, coefficient of variation;

\* Significance correlation at  $\alpha \leq 0.05$ .

\*\* Significance correlation at  $\alpha \leq 0.01$ .

\*\*\* Significance correlation at  $\alpha \leq 0.001$ .

‡ Parameter estimate.



**Fig. 3.** Regression analysis between soil CO<sub>2</sub> flux, and microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) for different land uses at Zigui County.

$6.0 \pm 0.31$  and  $5.4 \pm 0.29$  ( $\pm$ S.D.)  $\text{Mg CO}_2 \text{ ha}^{-1} \text{ year}^{-1}$  for Q-1, Q-2, Z-1, Z-2, Q-3, Z-3 and Z-4, respectively.

### 3.5. Impacts of environmental variances on CO<sub>2</sub> fluxes

Unlike other studies, effect of environmental variables on CO<sub>2</sub> fluxes was not predicted separately in this study. We used one set of parameters including all the six variables (soil temperature, WFPS, MBC, MBN, DOC and DON) in one linear model of variation, so that these variables can show their effect in conjunction with each other.

Soil environmental factors such as soil temperature, WFPS, MBC, MBN, DOC, and DON, that affects microbial activities, are most important in terms of CO<sub>2</sub> emission. A significant model was

developed including all of the above mentioned predictors, having significant effects alone or in interaction terms. Soil CO<sub>2</sub> fluxes displayed pronounced dependency on soil temperature from all land-use types ( $R^2 = 0.45\text{--}0.78$ , Table 3). While no significant effect of WFPS on soil CO<sub>2</sub> flux was observed from all land-use types ( $R^2 = 0.007\text{--}0.25$ ,  $P > 0.05$ , Table 3). Compared to soil temperature and WFPS alone, soil temperature and WFPS significantly improved the model fit simultaneously. A positive T-WFPS interaction was observed for all land-use types ( $R^2 = 0.36\text{--}0.72$ , Table 3) except Z-2 and Q-3 which generally had similar magnitude of CO<sub>2</sub> flux (Fig. 2A).

Soil CO<sub>2</sub> flux responded positively to MBC in all land-use types ( $R^2 = 0.39\text{--}0.86$ , Table 3) except Z-3 (Fig. 3,  $P > 0.05$ ) where lowest WFPS was observed among all land-use types (Fig. 2C). Soil CO<sub>2</sub>

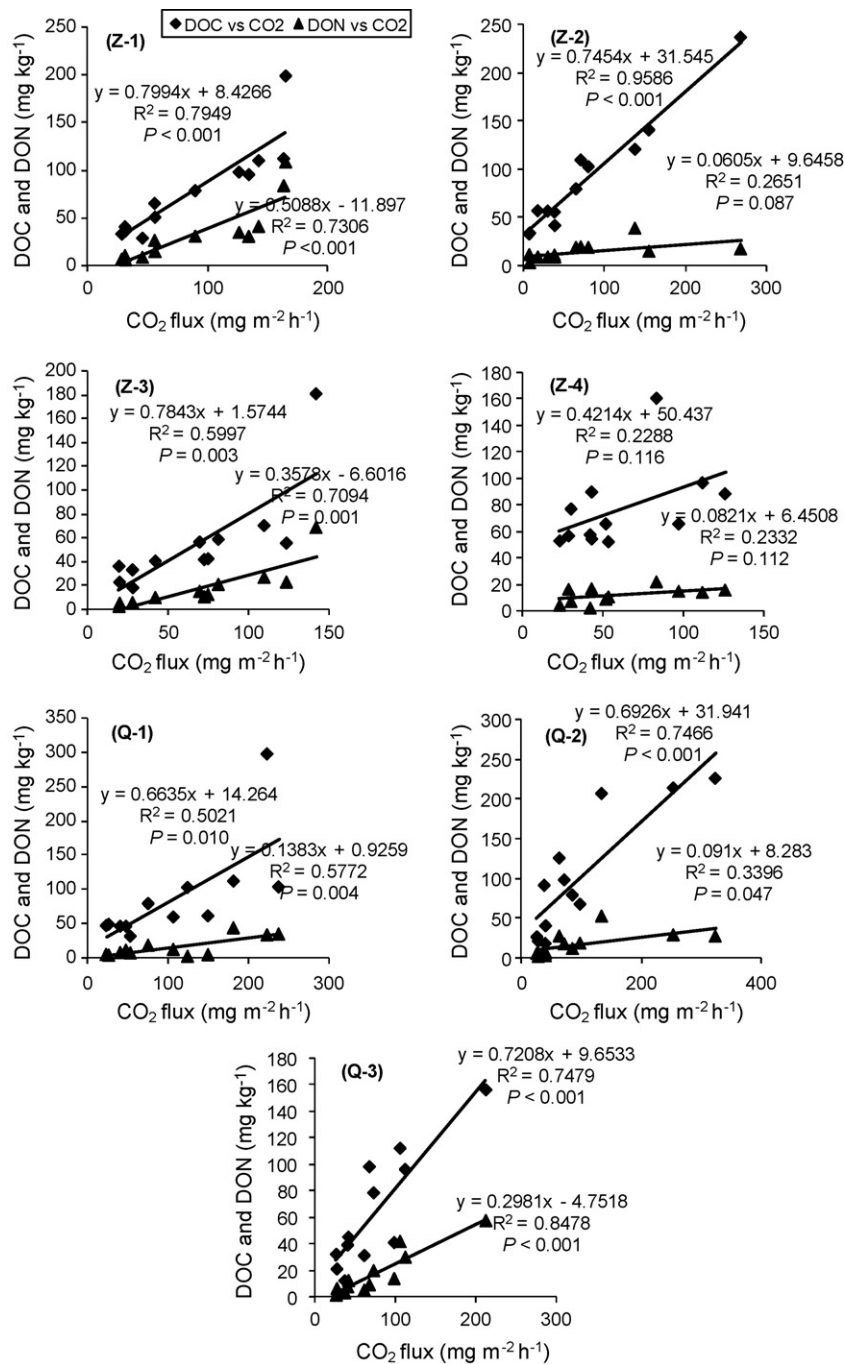


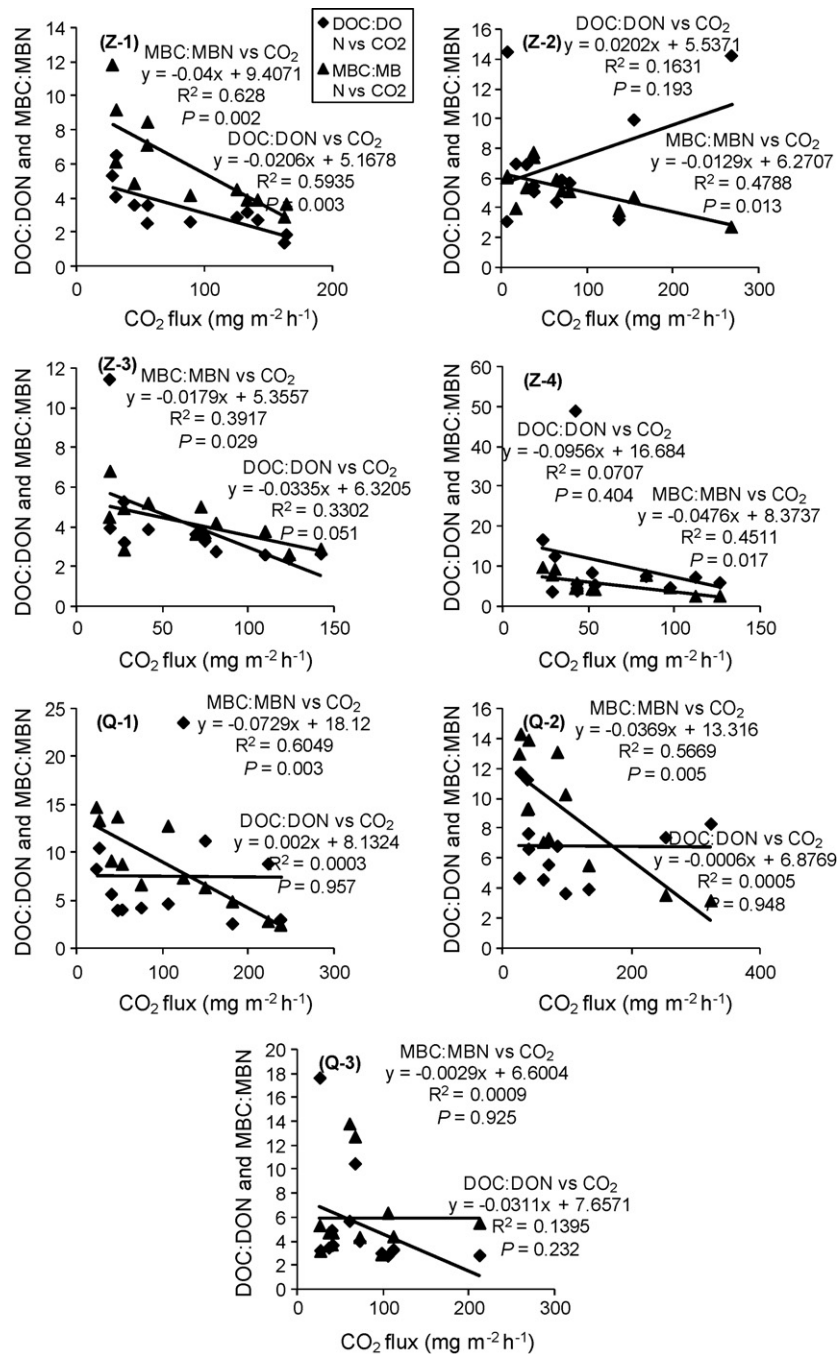
Fig. 4. Regression analysis between soil CO<sub>2</sub> flux, and dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) for different land uses at Zigui County.

fluxes were highly significantly correlated with MBN in all land uses ( $R^2 = 0.44\text{--}0.91$ , Table 3 and Fig. 3). The model provided a significant relationship between soil CO<sub>2</sub> flux and DOC contents in all land uses ( $R^2 = 0.50\text{--}0.95$ , Table 3 and Fig. 4) except Z-4. Likewise, some correlation between DON and CO<sub>2</sub> flux were found at site Z-1, Z-3, Q-1, Q-2 and Q-3 ( $R^2 = 0.34\text{--}0.85$ , Table 3 and Fig. 4), but no significant correlation existed at Z-2 and Z-4 site (Table 3).

To observe all the predictor's interactive effect on CO<sub>2</sub> flux, different reasonable combination of predictors were included in the model. As compared to every predictor's alone effect on CO<sub>2</sub> fluxes, the dependency (improvement/diminution) of predictor's interactive effect on soil CO<sub>2</sub> flux was different among sites. To some extent, the simultaneous effect of soil temperature and WFPS did showed their interaction with soil MBC and MBN (except Z-3), and

soil DOC and DON (except Z-4) to have an effect on CO<sub>2</sub> fluxes (Table 3). These interactive effects varied among different sites, either improved or diminished than every predictor's alone effect (Table 3). However, to exhibit the influence on CO<sub>2</sub> flux, a generally improved and significant interaction term was obtained with all of the above mentioned predictors together (Table 3) in all land uses. Furthermore, a whole regression model including all the predictors, accounted for 89–98% of soil CO<sub>2</sub> flux variability (Table 3) in all the land-use types.

Correlation between soil CO<sub>2</sub> flux, and MBC:MBN ratio, DOC:DON ratio (Fig. 5) and Qt was regressed separately. A significant correlation between soil CO<sub>2</sub> flux and MBC:MBN ratio was observed from all land-use types ( $R^2 = 0.39\text{--}0.63$ , Fig. 5) except Z-3. Unlike MBC:MBN ratio, DOC:DON ratio showed no consistent pat-



**Fig. 5.** Regression analysis between soil CO<sub>2</sub> flux, and microbial biomass carbon to microbial biomass nitrogen ratio (MBC:MBN) and dissolved organic carbon to dissolved organic nitrogen ratio (DOC:DON) for different land uses at Zigui County.

tern during the year, and was poorly correlated with soil CO<sub>2</sub> flux while excluding Z-3 (Fig. 5). The Q<sub>t</sub> value significantly correlated with soil CO<sub>2</sub> flux from all the land-use types except Z-3 where lower soil TOC was observed during the study period (Table 1 and Fig. 2D).

#### 4. Discussion

##### 4.1. Soil CO<sub>2</sub> fluxes

Regarding CO<sub>2</sub> fluxes, only a relatively few studies have been carried out in subtropical areas. Furthermore, to assess the impact of climate change around the area surrounding TGD, we measured

CO<sub>2</sub> fluxes from seven types of land uses including disturbed as well as undisturbed soils. The mean annual site CO<sub>2</sub> emission rates ranged from 5.4 to 9.5 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup>. The results found here fall within the range of soil CO<sub>2</sub> emission reported in similar subtropical areas (e.g. Lou et al., 2004; Tang et al., 2006; Iqbal et al., 2008).

Differences in the land-use types and topographic position seem to exert some controls on potential CO<sub>2</sub> fluxes from the soils. Our results agree with the fact that agricultural soils often have higher CO<sub>2</sub> emissions than soils under native vegetation. Iqbal et al. (2008) reported that conversion of woodland to agriculture land uses significantly increased the soil CO<sub>2</sub> emission (9.01, 7.27, 5.54 and 5.33 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup> for paddy, orchard, upland and woodland,

respectively). Conversion from secondary forest peat to paddy field increased the annual CO<sub>2</sub> emissions into the atmosphere (from 12.0 to 15.0 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup>) (Inubushi et al., 2003). In this study, soil CO<sub>2</sub> fluxes significantly differed among the seven soils under different land uses following the order of Q-1 > Q-2 > Z-1 > Z-2 ~ Q-3 > Z-3 > Z-4 ( $P < 0.001$ , Fig. 2A and Table 2). Furthermore, soil CO<sub>2</sub> flux was significantly higher from Q-3 (tilled orchard) as compared to Z-3 (non-tilled orchard). This was consistent with the findings of Wei et al. (2007) who reported tillage induced CO<sub>2</sub> fluxes in Northeast rice fields in China, as tillage is known to produce a temporary burst of CO<sub>2</sub> flux from soil surfaces (Rochette and Angers, 1999). A significant part of the effect of tillage on increased CO<sub>2</sub> emission may be linked to its direct impact on soil density and water content, and its indirect impact on oxygen levels, gas diffusion, and aeration. In this study, soil TOC was not found to be significantly correlated with soil CO<sub>2</sub> fluxes, which suggests that other relevant factors were responsible for controlling CO<sub>2</sub> emission from different land uses.

#### 4.2. Effect of seasonal changes on CO<sub>2</sub> fluxes

Soil CO<sub>2</sub> emission had clear seasonal fluctuations and was significantly higher in hot-humid season than in cool-dry season (Fig. 2A). The seasonal pattern of soil CO<sub>2</sub> flux was in accordance with the finding of Tang et al. (2006), who reported it to be coinciding with the seasonal changes of soil temperature and moisture. The same seasonal trend was observed by Liu et al. (2008). Iqbal et al. (2008) also found seasonal changes in DOC and soil temperature, which was found to be correlated with soil CO<sub>2</sub> flux. Lou et al. (2004) reported seasonal changes of CO<sub>2</sub> flux to be correlated with soil temperature, soil microbial carbon and DOC. However, in addition to soil temperature, soil moisture, MBC, and DOC, we also found soil CO<sub>2</sub> flux to be strongly correlated with precipitation (data not shown), MBN, and DON (Figs. 3 and 4, respectively). Furthermore, soil CO<sub>2</sub> flux was not only increased with SMB and DOS as found in this study, but it can also be related to fine root biomass as found in the tropical forests (Adachi et al., 2006). The higher CO<sub>2</sub> emissions during the hot-humid season can also be due to increased root respiration as result of root development with high soil temperature (around 30 °C) (Iqbal et al., 2008). The CV values of CO<sub>2</sub> fluxes in dry season (29.5–85.2%) were more variable than those in humid season (43.1–74.8%), which indicated a higher temporal variability in dry season than in humid season.

#### 4.3. Effect of environmental variables on CO<sub>2</sub> fluxes

##### 4.3.1. Soil temperature and moisture

In this study, soil temperature exhibited distinctive positive correlation with soil CO<sub>2</sub> fluxes (Table 3), which was in well agreement with the results from previous studies (e.g. Tang et al., 2006; Iqbal et al., 2009b). However, no significant correlation between WFPS and CO<sub>2</sub> flux was found in all land-use types (Table 3), which was in well agreement with the findings of Iqbal et al. (2008), who reported a negative correlation for paddy-upland and woodland soils from a nearby location at Xianning. These results indicate that soil temperature was an important variable in modeling annual CO<sub>2</sub> release, and WFPS was not so. While additional variables were needed when modeling the temporal variations of soil CO<sub>2</sub> emission.

##### 4.3.2. Microbial biomass and quotient

SMB (MBC, MBN) is another important factor that might have an impact on soil CO<sub>2</sub> production, as it is considered a sensitive indicator of soil quality and is closely related to soil fertility (Wardle et al., 1999). SMB has been shown to effect soil CO<sub>2</sub> fluxes (e.g. Singh et al., 2008). However, in this study, we separated the SMB into its two components i.e. MBC, MBN, to see whether these can have the same

effect when regressed separately. The monthly variations in soil CO<sub>2</sub> emission followed the large fluctuations in MBC (except Z-3) which had marked seasonal fluctuations in all land uses. This was in contrast with the findings of Lou et al. (2004), where no significant correlations between soil CO<sub>2</sub> flux and MBC were observed, as MBC content had no seasonal fluctuations in Yingtan, subtropical soils. However, soil CO<sub>2</sub> fluxes and the quantity of MBC were significantly correlated and differed between three red soils in Yingtan, similar to those observed in our study ( $R^2 = 0.73$ ,  $P < 0.001$ , data not presented). This suggests that, in addition to sampling date variations of CO<sub>2</sub> flux with MBC, the quantity of MBC differed among every land-use change, and in turn, controlled the dynamics of CO<sub>2</sub> emission. Like MBC, the similar effects of MBN with soil CO<sub>2</sub> flux were observed except that as compared to MBC, CO<sub>2</sub> flux had pronounced dependency on MBN in all land uses (Table 3). Furthermore, the quantity of MBN significantly correlated with CO<sub>2</sub> flux among different land uses ( $R^2 = 0.53$ ,  $P < 0.001$ , data not shown). This indicates that a shift in MBN provided a shift in CO<sub>2</sub> emission from different land uses, which may be due to the different composition of the substrate, turn over of roots or organisms, or organic or inorganic fertilizer input into the soil. It was notable to see that soil MBC and MBN did not follow a trend similar to that observed for soil TOC in all land uses (Fig. 2D, E and F). Even soil TOC was not significantly correlated with soil CO<sub>2</sub> flux (data not shown), which shows that it is the quality, not the quantity of soil organic matter which controlled the CO<sub>2</sub> flux in different land cover. Furthermore, MBC:MBN ratio significantly correlated with soil CO<sub>2</sub> flux (Fig. 5) which confirm that the quality of soil organic matter determines the soil CO<sub>2</sub> emission, and along with MBC, MBN should also be included for CO<sub>2</sub> modeling in different land uses.

To further assess the quality of soil TOC, microbial quotient ( $Q_t$  = the ratio of MBC to TOC) was calculated, as it is regarded by some authors as a good index of the changes in SOM quality (Insam and Merschak, 1997). Even, little is known about the quality of organic matter inputs under different land uses i.e. from vegetable field to pine forest. In this study,  $Q_t$  significantly correlated with soil CO<sub>2</sub> flux in different land uses (data not shown), which shows that despite of the different quantities of substrates in different land uses (Table 1 and Fig. 2D), microbial activity is a major factor regulating the seasonal variation of CO<sub>2</sub> fluxes in different land uses. However, a poor correlation between  $Q_t$  and CO<sub>2</sub> flux was observed from Z-3, which had relatively lower TOC than other land uses. This suggests that, to some extent, quantity of TOC might be responsible for soil CO<sub>2</sub> emissions (Degens, 1998).

##### 4.3.3. Dissolved organic substances

Among the factors associated with soil CO<sub>2</sub> flux, DOS (DOC, DON) could be considered as the primary factors regulating the seasonal variation of SMB, which in turn, controls the CO<sub>2</sub> emission from the soil. DOC has been proposed as an indicator of the C availability to soil microorganisms (Boyer and Groffman, 1996). It is assumed that all the dissolved substances are labile and utilized rapidly (e.g. Burford and Bremner, 1975). So, there is often a reasonably good correlation found between the concentration of DOC and soil CO<sub>2</sub> flux (e.g. Lou et al., 2004; Iqbal et al., 2008). In this study, significant relationships were found between soil CO<sub>2</sub> flux and DOC in different land uses (Table 3 and Fig. 4). However, in spite of seasonality, no significant correlation was observed between CO<sub>2</sub> flux and DOC in Z-4, which suggests that the C substrate in Z-4 was not enough suitable for mineralization to influence CO<sub>2</sub> emission. The quantity of DOC also significantly correlated with CO<sub>2</sub> flux among different land uses ( $R^2 = 0.26$ ,  $P = 0.017$ , data not shown). DON represents a significant pool of soluble N in most ecosystems, and previous studies have shown that DON concentrations may show little seasonality (Willett et al., 2004), consistent with the results observed in this study. However, to our knowledge, its relationship with soil

CO<sub>2</sub> fluxes is still unknown. In this study, the correlation between DON and soil CO<sub>2</sub> fluxes differed in different land uses. Some correlations between DON and CO<sub>2</sub> flux were found at site Z-1, Z-3, Q-1, Q-2 and Q-3 (Table 3 and Fig. 4). However, no significant correlations were observed at Z-2 and Z-4 sites (Table 3). Although DON enter the soil from a range of sources including dry and wet deposition, litterfall, root and microbial exudation, turnover of roots and organisms, urine and feces, and organic fertilizer additions to soil (Kalbitz et al., 2000). Of these sources, root exudation and turnover are likely to be the most dominant in many agricultural systems particularly where inorganic fertilizers are added (Christou et al., 2006). However, in this study, although different amount of nitrogen fertilizers were used among different land uses. Neither DON nor CO<sub>2</sub> fluxes correlated with amount of nitrogen fertilizer applied (data not presented). This indicates that neither DON nor soil CO<sub>2</sub> fluxes were influenced by sole applications of inorganic nitrogen fertilizer, but it might be root exudation and turnover from different vegetation across different land uses (Christou et al., 2006) which may be partial sources for DON and in turn, regulated the soil CO<sub>2</sub> flux. However, the quantity of DON was not correlated with CO<sub>2</sub> flux among different land uses ( $R^2 = 0.03$ ,  $P = 0.436$ , data not shown). This suggests that DON was only responsible for the sampling date variation of CO<sub>2</sub> fluxes. Unlike MBC:MBN ratio, DOC:DON ratio did not show consistent pattern during the year, and was poorly correlated with soil CO<sub>2</sub> flux while excluding Z-3 (Fig. 5). This shows that DOC:DON ratio was not directly involved in soil CO<sub>2</sub> emission, but it was MBC:MBN ratio which might be influenced by DOC and DON in the soil, and in turn, significantly influenced the soil respiration.

#### 4.4. Predominating factors affecting soil CO<sub>2</sub> flux

The effects of soil temperature and WFPS on soil CO<sub>2</sub> fluxes are well known and have been demonstrated in numerous field studies (e.g. Iqbal et al., 2008). In this study, a strong correlation between soil temperature and CO<sub>2</sub> fluxes was observed in all land uses (Table 3). However, poor relationship between WFPS and CO<sub>2</sub> fluxes was observed in all land uses (Table 3). WFPS can also affect the magnitude of soil CO<sub>2</sub> emissions as well as its response to soil temperature due to the interaction between WFPS and soil temperature (Fang and Moncrieff, 2001). A significant  $T \times$  WFPS interaction was found in different land uses (Table 3), which was in well agreement with Tang et al. (2006), who observed a highly positive correlation between soil CO<sub>2</sub> flux and soil temperature  $\times$  WFPS interaction in subtropical Dinghushan Nature Reserve. The coefficient of determination from our multiple regression model including soil temperature and WFPS was 36–72%. This was considerably less than the coefficient of determination of 69–85% reported for soil CO<sub>2</sub> flux in Dinghushan Nature Reserve (Tang et al., 2006). The study in Dinghushan Nature Reserve included WFPS and temperature variables and their interaction, regressed separately. While in this study, these variables and their interaction were regressed simultaneously in one model of variation, to show their relative effects in conjunction with each other. In this study, however, no significant correlation between soil CO<sub>2</sub> flux and soil temperature  $\times$  WFPS interaction was observed from Z-2 and Q-3 sites. We were not able to find any suitable reason for that, but it was interesting to note that both of these sites have almost similar magnitude of WFPS and CO<sub>2</sub> flux (Fig. 2A and C). In summary, from both of the above mentioned factors, soil temperature had a more marked impact on soil CO<sub>2</sub> flux (Table 3), which agrees well with our previous findings (Iqbal et al., 2008). This indicates that soil temperature was a major factor influencing soil respiration in areas with small ranges of seasonal changes in WFPS.

Including MBC and MBN in the regression equation improved or displaced part of the temperature effect that is intimately linked with the soil CO<sub>2</sub> flux, since the general temperature trend is similar

to that of soil CO<sub>2</sub> flux (Iqbal et al., 2008). The coefficient of determination for Z-1, Z-3, and Q-1 was reduced from 61 to 35%, 74 to 18%, and 78 to 66%, following inclusion of MBC and MBN. While for Z-2, Z-4, Q-2, and Q-3, it was increased from 47 to 80%, 62 to 78%, 69 to 84%, and 45 to 70%, respectively, following inclusion of MBC and MBN. This indicates that the MBC and MBN had significant interactions with soil temperature to show their mutualistic effect on soil CO<sub>2</sub> flux, since MBC and MBN are known to be well influenced by soil temperature (Feng et al., 2009; Steenwerth and Belina, 2008). Therefore, with the anticipated global warming, rising temperature can lead to higher physiological activities of soil microorganisms, and in turn to higher decomposition rates and soil respiration (Zhou et al., 2007). Although DOC and DON had significant seasonal variability (Fig. 2G and H), including DOC and DON in the regression equation did not improve the regression fit compared to the soil temperature while excluding Z-2 and Q-3 (Table 3). This occurred since soil temperature is not well known to be strongly correlated with DOC and DON, but the agronomic events (e.g. onset of irrigation and plowing) which control the concentrations of DOC and DON (Christou et al., 2006). Our results were consistent with those of Iqbal et al. (2008) who found a decrease in coefficient of variation, after inclusion of DOC in the multiple regression equation.

When all the variables (soil temperature, WFPS, MBC, MBN, DOC, and DON) were included in the multiple regression equation, improved/reduced regression fit was obtained among different land uses with the same trend as described following inclusion of MBC and MBN (Table 3). This indicates that MBC and MBN were the dominant factors regulating soil CO<sub>2</sub> flux. However, when all the variables were included in the multiple stepwise regression analysis, among all variables, different trend of dominancy was observed in all land uses as: soil temperature for Z-3 and Q-1, MBC for Z-4, MBN for Q-2, DOC for Z-1 and Z-2, DON for Q-3 (data not shown). Since, the soil temperature has been regarded as the dominating factor controlling soil CO<sub>2</sub> flux (Lou et al., 2004), our results indicate that, apart from soil temperature, MBC, MBN, DOC, and DON exert strong controls on soil CO<sub>2</sub> flux. This was in contrast with the finding of Iqbal and Ronggui (2009), who reported that soil temperature was the dominant variable in controlling soil CO<sub>2</sub> flux while excluding all the other variables. Furthermore, a whole regression model including all the predictors, accounted for 89 to 98% of soil CO<sub>2</sub> flux variability (Table 3) in all land-use types. The presence of strong statistical relationships between soil CO<sub>2</sub> fluxes and environmental factors is probably because of inclusion of all the environmental variables in one model of linear regression (Franzleubbers et al., 2002; Iqbal et al., 2008), because the soil environment is quite complicated and involves a lot of variables which are always interacting with each other. In summary, the results presented in this study indicates that for estimating the future soil-to-atmosphere C flux, apart from the soil temperature, microbial biomass and dissolve organic substances must be considered in a warming future as these can explain a major part of temporal variation of soil CO<sub>2</sub> fluxes.

## 5. Conclusion

Agricultural soils in Tropic of Cancer are important global sources of CO<sub>2</sub> emissions. Since CO<sub>2</sub> fluxes vary by orders of magnitude in time and space, depending upon land use and management, rainfall, soils and carbon input, it is clear that more information is needed that can explain major part of temporal variation of CO<sub>2</sub> fluxes in different land uses. Therefore, this study was conducted to explain the quality and quantity of substrate, and the environmental variables with relation to CO<sub>2</sub> emission in different land uses in a subtropical region.

Soil CO<sub>2</sub> fluxes were significantly different among different land uses, being higher for agriculture land uses than pine forest soil.

Even significant differences were observed among agriculture land uses. Different quantities of MBC and MBN significantly regulated the CO<sub>2</sub> emission among different land uses, relatively weakly regulated by DOC, while not being regulated by DON. This indicates that there was a significant shift in MBC and MBN which caused to induce a shift in CO<sub>2</sub> emission among different land uses.

Soil CO<sub>2</sub> fluxes were significantly correlated with Q<sub>t</sub> and MBC:MBN ratio, while not being correlated with TOC, which indicate that the quality, not the quantity of substrate was responsible for soil-to-atmosphere emission of CO<sub>2</sub>, as different carbon inputs from different land uses supports different amounts of microbial biomass per unit of substrate. The relationship between CO<sub>2</sub> fluxes and all the concerned variables were presented in one linear model of variation. The primary advantages of this model were that, we used a single set of parameters to predict soil CO<sub>2</sub> fluxes, which can be used to explain a large percentage of the temporal variability observed in soil CO<sub>2</sub> efflux rates from the agroforest ecosystem. As compared to CO<sub>2</sub> emission predicted by soil temperature and moisture, a generally improved and significant interaction term was obtained with all of the predictors together, explaining 89–98% of soil CO<sub>2</sub> flux variability in all land uses. This indicates that to improve the predictions of soil-to-atmosphere CO<sub>2</sub> emissions, it appears necessary to include SMB and DOS in the model. Furthermore, when all the variables were included in the multiple stepwise regression analysis, among all variables, different trend of dominance was observed in all land uses as: soil temperature for Z-3 and Q-1, MBC for Z-4, MBN for Q-2, DOC for Z-1 and Z-2, DON for Q-3. This suggests that, apart from soil temperature, SMB and DOS should also be included for estimating the soil CO<sub>2</sub> fluxes for different land uses probably at global and especially at the regional scale. However, it seems that carbon cycling responses to environmental change can be highly ecosystem-specific (e.g. with rainfall pattern), and thus needs to be verified across different ecosystems.

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