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Excessive Nitrogen Inputs in Intensive Greenhouse Cultivation May Influence Soil Microbial Biomass and Community Composition

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Abstract: Intensive greenhouse vegetable-production systems commonly utilize excessive fertilizer inputs that are inconsistent with sustainable production and may affect soil quality. Soil samples were collected from 15 commercial greenhouses used for tomato production and from neighboring fields used for wheat cropping to determine the effects of intensive vegetable cultivation on soil microbial biomass and community structure. Soil total nitrogen (N) and organic-matter contents were greater in the intensive greenhouse tomato soils than the open-field wheat soils. Soil microbial carbon (C) contents were greater in the greenhouse soils, and soil microbial biomass N showed a similar trend but with high variation. The two cropping systems were not significantly different. Soil microbial biomass C was significantly correlated with both soil total N and soil organic matter, but the relationships among soil microbial biomass N, soil total N, and organic-matter content were not significant. The Biolog substrate

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utilization potential of the soil microbial communities showed that greenhouse soils were significantly higher (by 14%) than wheat soils. Principal component (PC) analysis of soil microbial communities showed that the wheat sites were significantly correlated with PC1, whereas the greenhouse soils were variable. The results indicate that changes in soil microbiological properties may be useful indicators for the evaluation of soil degradation in intensive agricultural systems.

Keywords: Biolog substrate utilization, excessive fertilizer inputs, greenhouse production, soil microbial biomass

INTRODUCTION

Vegetable production in China increased 79.5% by 2005 compared to 1995 and 16.9% compared to 2000 because of accelerating economic development (National Bureau of Statistics of China 2006). During this period, some of the area devoted to cereal production was converted to vegetable production and especially to intensive cultivation in plastic greenhouses. Intensive greenhouse vegetable production usually involves fertilizer application rates in excess of crop requirements, and these systems are not sustainable in the long term. In Shouguang, a typical greenhouse vegetable production region in Shandong Province, northern China, excessive fertilizers are commonly combined with organic manure applications to give total nitrogen (N) inputs of typically $>1000 \text{ kg N ha}^{-1}$ for maximum yields of vegetables (Ju et al. 2004). Nitrogen-use efficiency in such crops is typically only about 10%, resulting in high risk of environmental pollution and degradation of soil quality (Zhu et al. 2005). There is considerable concern about diffuse environmental pollution from these vegetable production systems because of nitrate pollution of groundwater and nitrous oxide emissions to the atmosphere from the soils (He et al. 2007). It is well known that fertilizers and manures can exert various effects on soil physical and chemical properties (Chen et al. 2004). However, there is little information available on the effects of excessive fertilizer and manure inputs on microbial properties in soils in which cereal cropping has been replaced with greenhouse vegetable production in northern China.

Increasing emphasis has recently been given to the effects of farming systems on soil quality (Mäder et al. 2002) as reflected in various biological soil characteristics such as soil microbial activity, biomass, and community structure. The importance of microorganisms in soil fertility and productivity has been increasingly recognized (Nannipieri et al. 2003). Microorganisms, because of their capability to respond quickly to environmental changes, might be expected to be efficient bioindicators (Avidano et al. 2005). Measurement of microbial biomass has been used

in studies on carbon (C) flow, nutrient cycling, and plant productivity (Ladd and Foster 1988; Doran and Zeiss 2000; Wang et al. 2004). Microorganisms responsible for the decomposition and mineralization of the organic fraction use part of the compounds contained in the residues as sources of nutrients and energy for biomass formation. Measurement of microbial biomass C and nitrogen (N) offers a means of assessing the response of the total microbial population to changes in agricultural management (Gupta et al. 1994; Witt et al. 2000). Numerous studies have shown that changes in the size and activity of the soil microbial community are major contributors to soil degradation caused by agricultural management (Dominy and Haynes 2002; Widmer et al. 2006). It is generally believed that direct measurements of functional diversity of soil microbial communities are likely to provide information more relevant to the functioning of soils than measurements of species diversity. The metabolic profile obtained using the Biolog assay provides a qualitative physiological fingerprinting of the potential functions of the microbial community (Garland and Mills 1991; Giller et al. 1997; Toyota and Kuninaga 2006). Although the Biolog technique is restricted to culturable microorganisms and does not provide an accurate assessment of the functional properties of microbial communities in soil (Preston-Mafham, Boddy, and Randerson 2002), it has been applied successfully in comparative soil analyses and has been shown to be a powerful and sensitive low-cost analytical tool for demonstrating differences or changes in soil microbiological characteristics (Widmer et al. 2006). Various studies have characterized the influence of chemical inputs on the functional and structural diversity of the microbial community (Larkin 2003; Fliessbach and Mäder 2004). However, the effects of excessive N inputs in vegetable production systems on the soil microbial community in the field and their attendant functions have not been fully elucidated.

The increasing area of vegetable production in China has led to greater concern about possible changes in the microbiological properties of soils. In the present study, we sought to compare soil microbial biomass and community structure in soils from open-field wheat cultivation and neighboring plastic greenhouse tomato cultivation in the same soil types several years after conversion from cereal production to vegetable production in order to investigate the microbiological characteristics of soils potentially subjected to degradation and environmental risk.

MATERIALS AND METHODS

The sampling sites were located in Shouguang, a typical intensive greenhouse vegetable production area in Shandong Province with a

typical continental monsoon climate. The annual average air temperature and precipitation from 1993–2003 were 12.4 °C and 558 mm. Fifteen typical open-field wheat (*Triticum aestivum* L.) and neighboring (within 5 m) plastic greenhouse tomato (*Lycopersicon esculentum* Mill.) cultivation sites were randomly selected (Table 1).

Every site was sampled in January 2007. A representative soil sample was obtained from each site by collecting 10–12 randomly selected cores 25 mm in diameter from every plot to a depth of 30 cm following a “W” path within the interrow areas inside and outside of the greenhouses. The cores from greenhouses were pooled, hand-picked to remove large pieces of detritus including plant materials and earthworms, sieved moist to pass a 2-mm mesh, mixed, and subsampled prior to analysis. After quartering of the composite samples, some subsamples were used fresh and analyzed at field moisture, and others were stored in airtight plastic bags at 4 °C prior to analysis. Organic C was determined by the dichromate method with an external heating procedure, and total N was measured by dichromate oxidation and a Kjeldahl procedure.

Both microbial biomass C and N were determined using a modified chloroform–fumigation–extraction method with fumigation. Briefly, fresh soil samples equivalent to 20 g oven-dry basis were weighed into 100-mL glass beakers and exposed to alcohol-free chloroform (CHCl₃) vapor in a vacuum desiccator containing soda lime at 25 °C for 24 h (i.e., fumigated). After removing the CHCl₃, C and N were extracted from the fumigated and nonfumigated samples with 0.5 M potassium sulfate

Table 1. Location and period after change in cultivation system of the 15 field sampling sites

Site	Latitude	Longitude	Years after change from wheat to tomato
1	36° 55' 23.6" N	118° 44' 41.2" E	7
2	36° 55' 22.8" N	118° 44' 44.8" E	7
3	36° 55' 24.6" N	118° 44' 41.6" E	7
4	36° 55' 08.5" N	118° 48' 22.4" E	6
5	36° 55' 05.1" N	118° 44' 50.4" E	6
6	36° 55' 01.0" N	118° 44' 54.0" E	7
7	36° 55' 05.8" N	118° 44' 55.9" E	8
8	36° 53' 44.4" N	118° 42' 40.7" E	3
9	36° 54' 12.5" N	118° 42' 02.1" E	6
10	36° 54' 00.5" N	118° 42' 02.5" E	5
11	36° 54' 44.2" N	118° 41' 52.1" E	7
12	36° 54' 54.1" N	118° 41' 47.0" E	2
13	36° 55' 44.7" N	118° 42' 37.8" E	14
14	36° 56' 40.9" N	118° 42' 12.8" E	7
15	36° 55' 02.9" N	118° 45' 05.6" E	8

(K₂SO₄) for 30 min on a shaker (soil–solution at a 1:4 ratio). Filtered soil extracts were stored in a freezer at –20 °C prior to analysis. Organic C in the filtered extracts was determined by oxidation with dichromic acid. Total N was measured by the colorimetric nitroprusside method (Mulvaney 1996). The difference between C and N extracted from fumigated and nonfumigated samples was converted into C and N in the microbial biomass using the K_{EC} and K_{EN} factors with values of 0.45 (Joergensen and Brookes 1990) and 0.20 (Wu, Brookes, and Jenkinson 1996), respectively. Two separate K₂SO₄ extracts were prepared from each replicate of bulked soil and analyzed as described.

Community-level physiological profiles (Biolog) were constructed using Biolog[™] ECOplates (Biolog Inc., Hayward, CA, USA), which contain 31 different C sources. Three replicate subsamples of 5 g of the soils were suspended (1 h at 200 rpm, 4 °C) in 45 mL of sterile deionized water, and soil suspensions were allowed to settle for 2 min before the supernatants were diluted 10-fold to obtain a final dilution of 10⁻³. Each well of a plate was filled with 125 mL of the final dilution (Garland and Mills 1991). Three replicate substrate sets were used from each soil sample. Plates were incubated in the dark at 25 °C, and the optical density at 590 nm was read periodically (every 24 h). Individual absorbance values of the 31 single substrates were corrected by subtraction of the blank control value (raw difference; RD). The data were normalized by dividing the RD values by the respective average well color development (AWCD) values to minimize the effects of different inoculum densities (Verschuere et al. 1997).

All chemical and microbial analyses were performed in duplicate, and the mean values were used in further calculations. The t-test was used to test for significant differences between the sampling sites using the SPSS 11.0 software package, and normalized absorbance Biolog profiles were examined using principal component analysis (PCA).

RESULTS

Soil total N content varied substantially among the sampling sites, but the mean total N content of greenhouse tomato soils was significantly greater than that of the adjacent open-field wheat soils (Figure 1). More than 50% of the greenhouses received more than 1000 mg kg⁻¹ total N input to the tomato crops, but fewer than 14% of the wheat fields received these excessive N inputs. The average soil total N content of the greenhouse tomato soils was 35.2% more than that of the wheat fields, and some specific sites showed a 97% increase. Greenhouse soils converted to tomato production also had soil organic-matter contents that were greater; 44.8% on average more than wheat fields (Figure 1). As

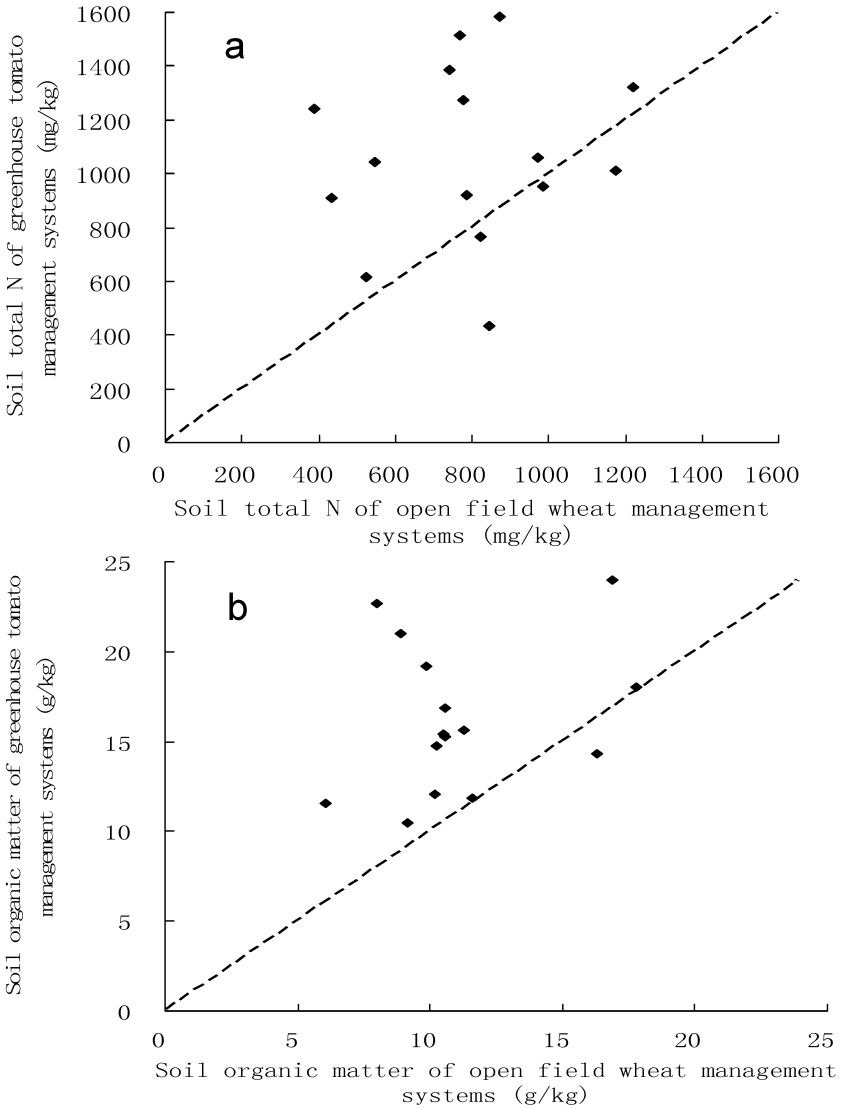


Figure 1. Scatter diagrams showing the relationships between (a) soil total N content in wheat and greenhouse tomato management systems ($p = 0.0131$ by paired-sample t-test) and (b) soil organic-matter content in wheat and greenhouse tomato systems ($p = 0.0007$). The dashed line shows the $X = Y$ relationship.

with soil total N, some specific sites had more than double the soil organic-matter content of wheat fields.

General trends in soil microbial biomass were similar to the trends in soil organic-matter content and soil total N. Microbial biomass C varied

between 101 and 326 mg kg⁻¹ in tomato greenhouse soils (Figure 2) and was significantly more than in wheat fields. Mean soil microbial biomass C was 61% greater in tomato greenhouses than in wheat fields. There was large variation in soil microbial biomass N content in both the greenhouse and wheat management systems, and in contrast to microbial biomass C, there was no significant difference between the two production systems (Figure 2).

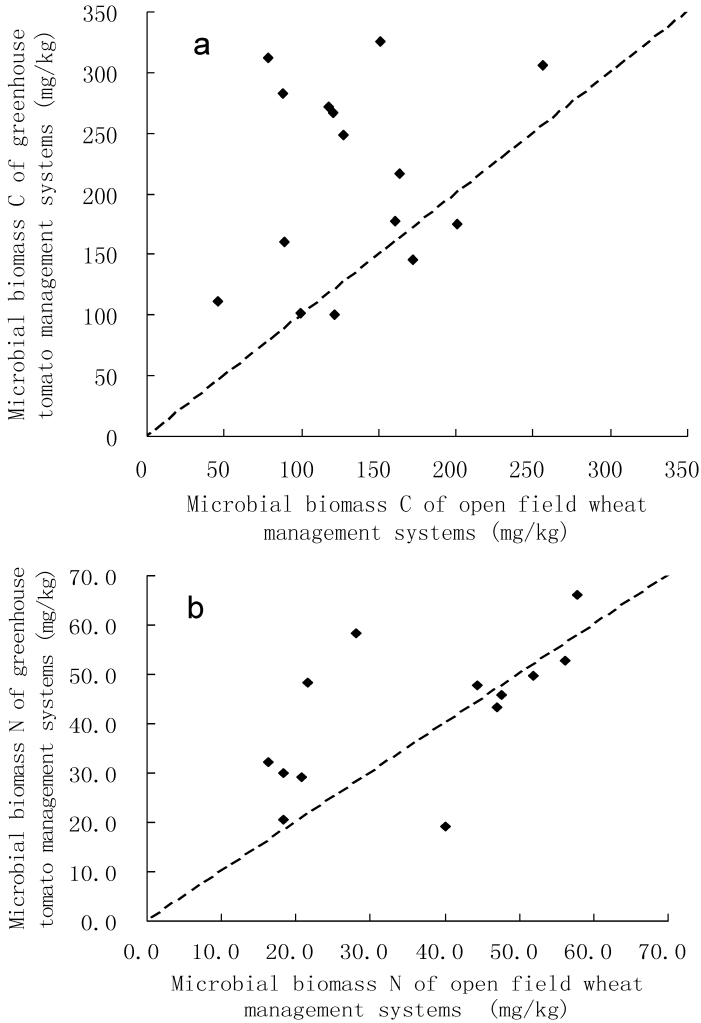


Figure 2. Scatter diagrams showing the relationships between (a) soil microbial biomass C in greenhouse tomato and open-field wheat management systems ($p = 0.0007$ by paired-sample t-test) and (b) soil microbial biomass N in greenhouse tomato and wheat ($p = 0.3856$). The dashed line shows the $X = Y$ relationship.

Linear regression analysis showed that microbial biomass C was significantly correlated with total N content of the soils sampled both inside and outside the greenhouses (Figure 3), and microbial biomass C was similarly correlated with soil organic-matter content. No such relationship was found between microbial biomass N with either soil total N content or soil organic matter.

The C substrate utilization patterns (defined as the number of C substrates utilized) shown by the microbial communities from the two different cultivation systems were determined using Biolog ECOplates. The number of substrates used by each community increased with incubation time until a plateau was reached at 240 h, and the metabolic rates reached their maximum values at 72 h (data not shown).

Differences in patterns of C use between the two cultivation systems were most significantly expressed by the differential rates of AWCD at 72 h (Figure 4). The trends of the indices were similar to those of microbial biomass. Greenhouse tomato soils had 14% greater values than wheat soils (a statistically significant difference) and followed similar patterns to soil microbial C. There were similar significant positive linear correlations between microbial biomass C and AWCD (Figure 5).

Principal component analysis of the AWCD data (Figure 6) did not show a very clear separation of the two different cultivation land uses on the plane of the first two components, PC1 and PC2, which accounted for 32% and 15% of the variance in the data. The general trends showed that

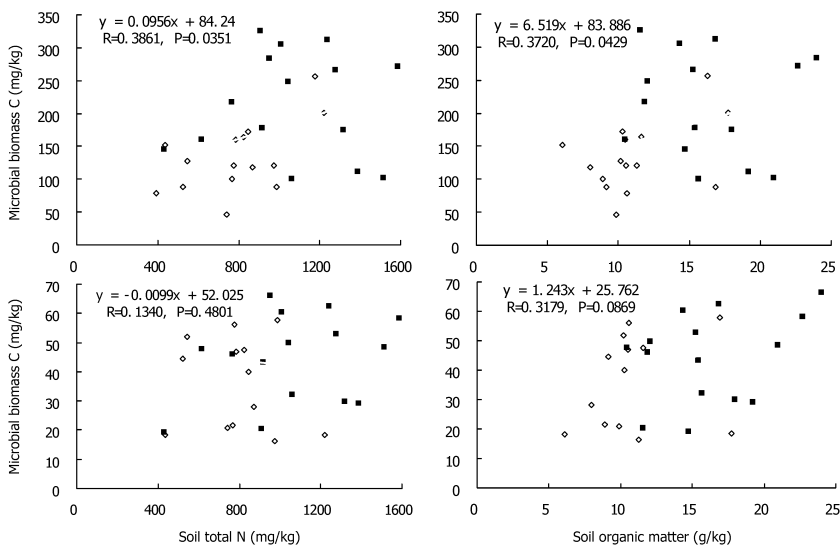


Figure 3. Scatter diagrams showing the relationships between soil microbial biomass and various soil chemical properties in greenhouse tomato (■) and wheat (◇) management systems.

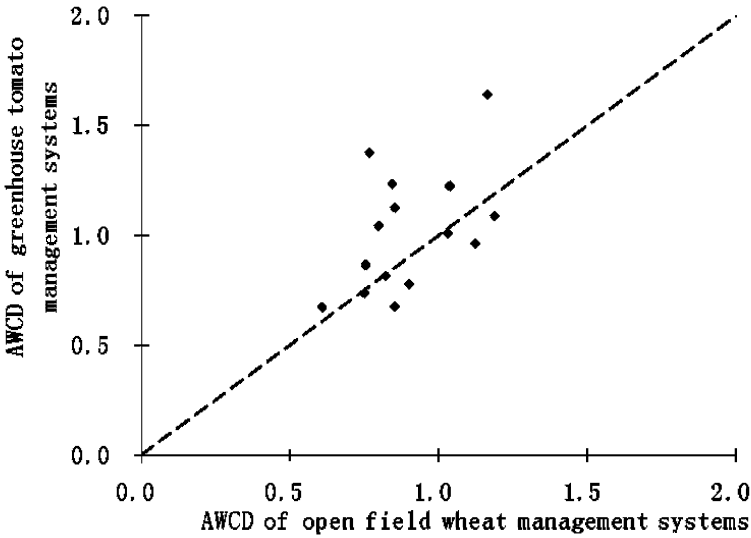


Figure 4. Relationship between average well color development (AWCD) values of substrate utilization patterns using Biolog ECOplates ($OD_{590\text{ nm}} \text{ well}^{-1}$) after 72 h of incubation in greenhouse tomato and wheat management systems ($p = 0.02765$) by paired-sample t-test. The dashed line shows the $X = Y$ relationship.

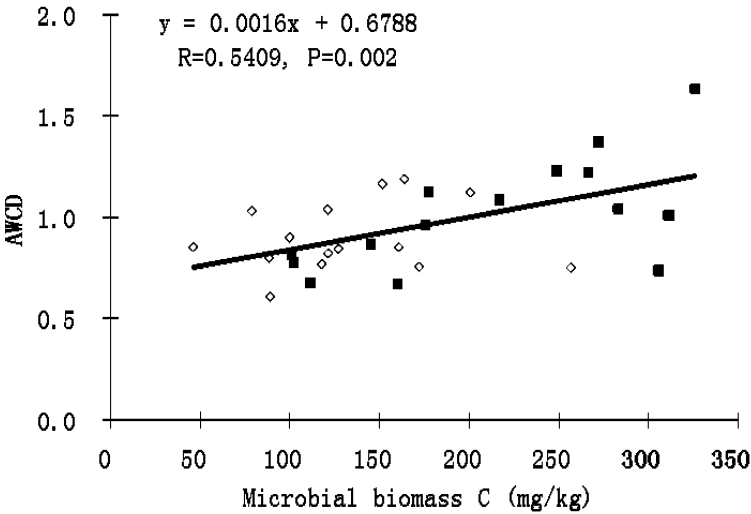


Figure 5. Relationship between soil microbial C and average well color development (AWCD) value in greenhouse tomato (■) and wheat (◇) management systems.

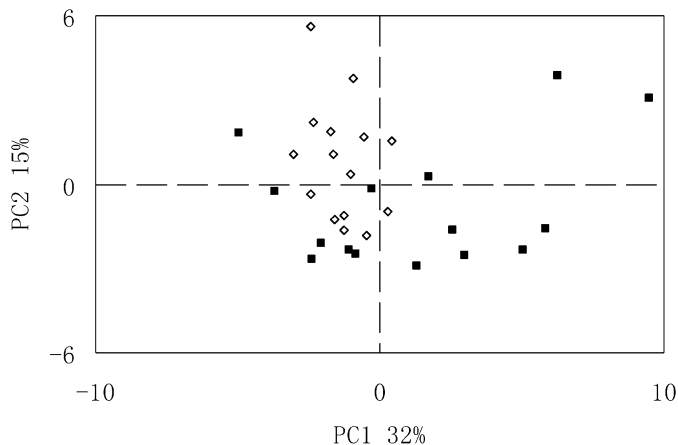


Figure 6. Principal component analysis of substrate utilization patterns using Biolog ECOplates ($\text{OD}_{590\text{nm}} \text{ well}^{-1}$) after 72 h of incubation in greenhouse tomato (■) and wheat (◇) management systems.

the wheat sampling sites were significantly correlated with PC1 at both sites, indicating a shared ability to utilize some substrates. However, there was some overlapping of the two systems. A large, seemingly random subset of greenhouse tomato soils appeared to be related to the separation of the sampling sites.

DISCUSSION

The soil total N contents of greenhouse tomato soils were significantly more than those of the adjacent wheat fields, and the average value was 1069 mg kg^{-1} , possibly resulting from the excessive inputs of fertilizer N and organic manures commonly applied in conventional intensive vegetable production systems in this part of northern China. The data indicate that soils in intensive greenhouse production systems have greater biomass N and show changes in microbial functional diversity compared with the adjacent cereal soils from which they are derived.

The selection of sampling sites was a critical aspect of our experimental design because it was necessary to compare the greenhouse soils after a number of years of vegetable production with adjacent soils with the same physicochemical properties. Nitrogen fertilizer application rates in this region range from about 500 to $1900 \text{ kg N ha}^{-1}$ and are increased dramatically by mixing poultry manure into the soil for each crop and growing two or more crops annually (Zhang et al. 1996; Zhu et al. 2005). The differences found in soil organic-matter content and total soil N may be explained as a consequence of this management regime

(Figure 1). The plants cannot make use all of the N applied, and this may lead to nitrate leaching from the soil profile and gaseous emission of nitrous oxide (N_2O) formed from the denitrification of nitrate (McNeal et al. 1995), both of which are N-loss processes resulting in environmental pollution.

In the present study, the greenhouse tomato production system promoted soil microbial C contents (Figure 2), but it is not clear which factors are most important in regulating this variation. Grayston et al. (2001) suggested that differences in plant species composition were likely to exert strong selective pressures on the soil microbial community through plant-specific changes in the quantity and variety of compounds lost through rhizodeposition and litter and root senescence. In our study, the influence of different cover plant species on microbial biomass C was significant (Figure 2). Although no effect of crop species on microbial biomass N was found, the microbial biomass N values showed more pronounced changes than did microbial biomass C (Figure 2). This may have been due to the microorganisms differing much more in their N content than in their C content (Jordan et al. 1995).

Some studies have shown significant effects of plant cover on soil microbial biomass C and N values at the time of sampling but no effect of N fertilization (Bucher and Lanyon 2005). Others have indicated that the soil microbial biomass is a more sensitive indicator of soil fertility than soil organic-matter content because it responds readily to changes in soil physical and chemical properties (Shibahara and Inubushi 1997). Our results indicate that microbial biomass C values were significantly affected by soil N contents and organic-matter contents (Figure 3). In general, studies on the influence of N fertilizer inputs on soil biological properties have produced contradictory results, with some showing increases in the size of the microbial biomass (Fraser, Haynes, and Williams 1994; Omay et al. 1997) and others reporting the opposite trend (Ladd et al. 1994; Bardgett, Wardle, and Yeates 1998). Similar significant positive linear correlations have been reported between microbial biomass C and soil organic-matter content in 13 diverse soils differing in management practices (Witter, Martensson, and Garcia 1993). In the present study, the long-term application of excessive chemical fertilizer and manure N may have caused the observed differences in soil biological properties. The dramatic variation found in soil microbial biomass N is difficult to explain. The determination of microbial biomass N is important for the quantification of N dynamics in agricultural ecosystems because the microbial biomass controls soil inorganic N availability and loss (Bucher and Lanyon 2005). However, in extremely intensive cropping systems with very large N inputs, the microbial biomass may make a much smaller contribution to the functioning of the soil N cycle.

The structural diversity of a bacterial community may be found to be a very sensitive indicator of reaction to environmental change by a shift in its composition. Biolog community-level substrate utilization analysis is a potential method to assess functional community shifts in soils subjected to different management practices (Buyer and Drinkwater 1997) or in soils under different plant species cover (Grayston et al. 1998). The AWCD, which reflects the substrates that can be utilized, was significantly ($P < 0.05$) greater in the greenhouse tomato system than in soils under wheat cultivation. The AWCD results indicated that the biomass C values were the most similar in the two cropping systems, thus supporting the soil microbial biomass as an indicator of the promotion of microbial metabolic properties (Toyota and Kuninaga 2006).

Although bacterial activity in solution culture may be quite different from that in soil and the substrates of the Biolog ECOplates may not accurately represent the substrates present in the field, Biolog estimations do provide an indication of the potential functional diversity of the bacterial community in the soil (Bossio and Scow 1995; Papatheodorou, Argyropoulou, and Stamou 2004). In the present study, differences were found in the substrate utilization potential of the two different cultivation systems. There were no significant differences at PC1 within the wheat cultivation system, indicating that both plant species and soil chemical properties play an important role in defining the types of microorganisms that are physiologically active. Previous studies have indicated that functional redundancy of soil microorganisms led to structural changes without corresponding functional changes (Buyer and Drinkwater 1997; Marschner, Kandeler, and Marschner 2003). These different responses may explain the shift in the soil microbial community structure of the greenhouse tomato production system in our study.

CONCLUSIONS

The change from open-field wheat cultivation to the intensive greenhouse tomato production system had profound effects on the soil microbial biomass. Excessive soil N contents appeared to increase soil microbial C but not soil microbial N. Soil organic matter showed trends similar to soil microbial C. Our findings suggest that the changes in management practices influenced microbial composition and that those changes in soil microbial biomass and microbial community profiles can be used as an indicator of changes in soil chemical properties. Further investigations will be required to elucidate the relationships between cropping systems and soil microbial activities.

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