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Original Communication

Proposal for a new soil fertility index (SOFIX) for organic agriculture and construction of a SOFIX database for agricultural fields

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ABSTRACT

The evaluation of fertility of agricultural soils should not only include the chemical properties but also biological properties. A simple and rapid evaluation method of soil fertility is proposed (soil fertility index; SOFIX) based on bacterial biomass, nutrient circulation activities, and chemical properties. Methods for estimating bacterial biomass, nitrogen circulation activity, and phosphorus circulation activity are developed. Using the new methods, a SOFIX database of upland agricultural fields is constructed. Based on the SOFIX database, methods for the enhancement of material circulation are also developed for an efficient organic agricultural system.

KEYWORDS: soil fertility index (SOFIX), bacterial biomass, N circulation activity, P circulation activity, SOFIX-based organic agriculture

1. INTRODUCTION

Chemical fertilizers have greatly contributed to the enhancement of crop production for meeting the increasing food needs over the last century [1]. But, apart from increasing the cost of cultivation, the use of chemical fertilizers often results in leaching and run-off of nutrients and emission of greenhouse gases [2, 3]. Leaching of nitrogen (N) and phosphorus (P) adversely affects soil microorganisms and causes water pollution [4]. A shift from chemical materials-based to organic materials-based agricultural system is therefore necessary to protect the environment from further deterioration.

Indicators of soil fertility can be categorized mainly based on physical, chemical, and biological factors (Fig. 1). Generally, in a chemical materials-based agricultural system, the chemical and physical factors are given priority over the biological ones. However, in the organic materials-based agricultural system, biological factors such as quantity of organic matter, microbial biomass, rate of decomposition of organic matter, nutrient circulation activities etc. are important for improving soil fertility.

Microorganisms play an important role in the decomposition of organic materials and circulation of nutrients such as C, N, P, and S in soil (Fig. 2) [5-7]. In addition, the microbial biomass itself contains a labile pool of nutrients which is potentially available to plants [8, 9].

The soil fertility index (SOFIX) was developed considering the importance of biological factors in soil fertility. SOFIX keeps soil bacterial biomass and their activity at the center of the soil fertility considerations. This article focuses on three soil parameters of SOFIX: total bacterial biomass, nitrogen circulation activity, and phosphorus circulation activity.

2. MATERIALS AND METHODS

2.1. Soil bacterial biomass

Soil bacterial biomass is one of the most important indicators of soil fertility. In this section, a new

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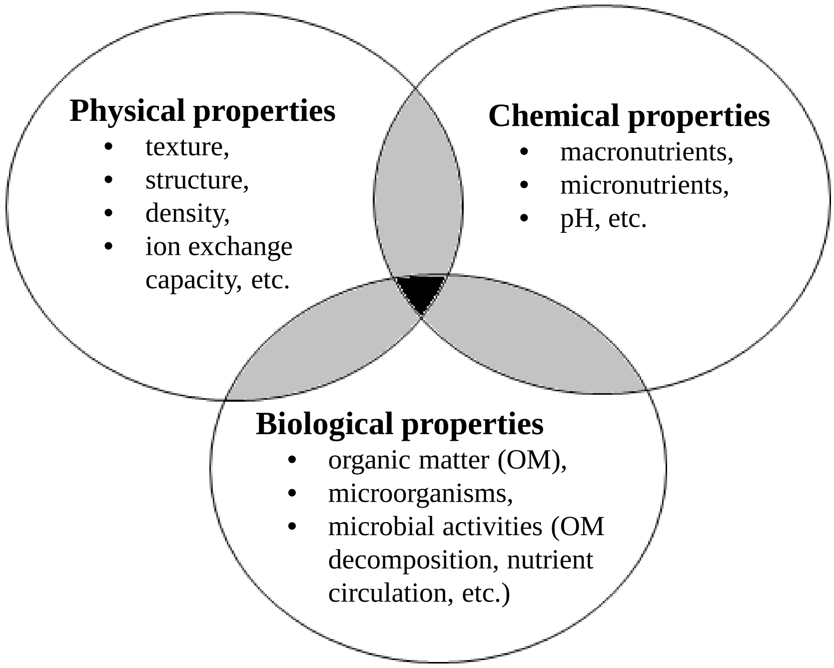


Fig. 1. Schematic representation of physical, chemical, and biological factors affecting soil fertility. White, grey, and black zones represent their importance for soil fertility in increasing order.

Fig. 2. Material circulation in soil environment.

method for the estimation of bacterial biomass is described.

Several methods have been developed to estimate the bacterial biomass in soil. Methods using agar plates with various growth media and culture conditions have previously been suggested for counting the number of microorganisms [10-12]. However, more than 99% of soil microorganisms do not grow on agar plates, rather they remain in a viable but not culturable (VBNC) state [10, 12-14]. Therefore, many studies have investigated ways to estimate the soil microbial biomass. For example, substrate-induced respiration (SIR) and chloroform fumigation extraction were developed based on microbial respiration [15, 16]. Similarly, a 4',6-diamino-2-phenylindole (DAPI) staining method was developed for counting soil microorganisms [17-22]. However many problem remain, such as complications associated with operation, quantification, reproducibility, and the high cost of the techniques.

Analysis of environmental DNA (eDNA) has been investigated as a new approach for estimating microbial biomass [23-28]. Since eDNA analysis does not require a cultivation procedure, this technique is useful for detecting VBNC microorganisms [26]. Several methods for extraction of eDNA from soil have been developed; however, the extraction efficiency and level of DNA damage require improvements.

To protect the eDNA from damage and quantify the amount through agarose gel electrophoresis, a slow-stirring method has been developed in our previous work [29]. The general protocol for extracting eDNA and estimating bacterial biomass is shown in Fig. 3. Using this method, the eDNA can be extracted efficiently with minimal damage from various kinds of soil (Fig. 4). The amount of eDNA and soil bacterial biomass exhibited linear relationship ($Y = 1.70 \times 10^8 X$; $r^2 = 0.96$), indicating that bacterial biomass could be evaluated by quantifying eDNA (Fig. 5).

2.2. Analyses of N and P circulation activities

Nitrogen and phosphorus are the two most limiting plant nutrients in soil for plant growth. The role of bacteria has been recognised for the supply of these two nutrients. A soil with an abundant bacterial biomass could improve the circulation activities of N and P. To enhance the productivity by the organic material-based agricultural system, methods to analyze N and P circulation activities were developed.

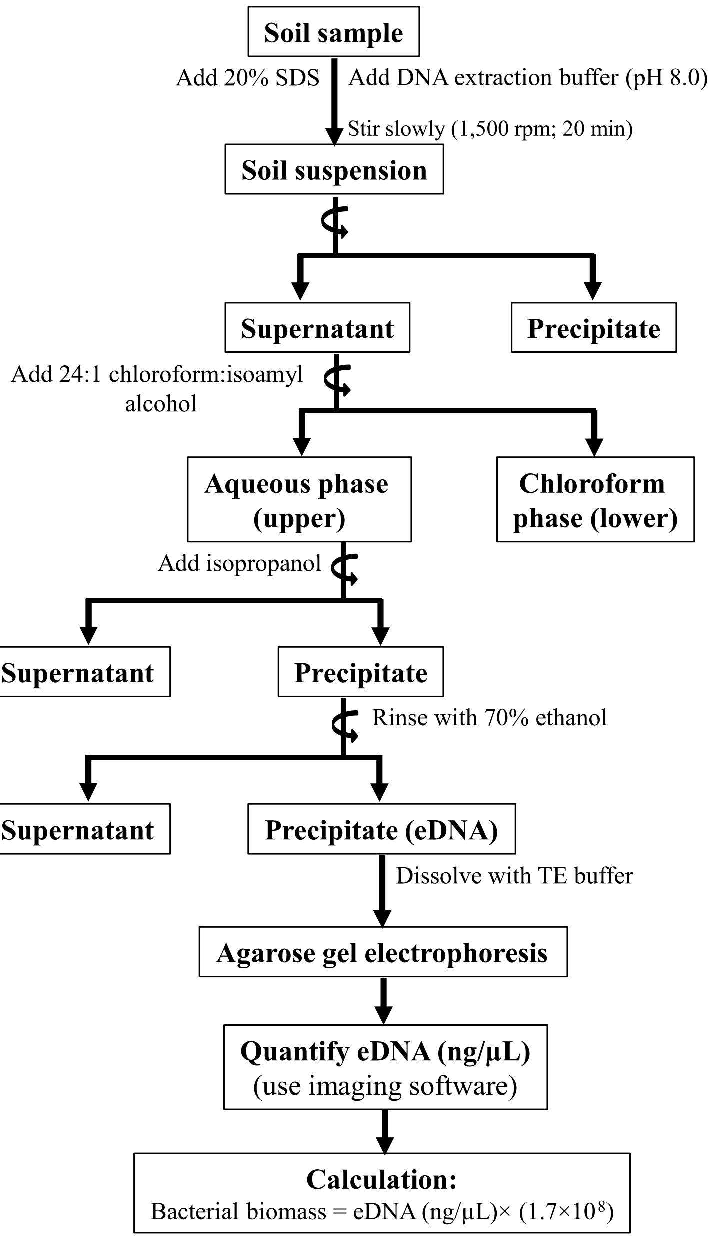


Fig. 3. Procedure for estimating bacterial biomass in soil.

2.2.1. Nitrogen circulation activity

Organic N (e.g., protein) is the most predominant source of N in soil. Soil microorganisms are involved in several steps of N circulation activity from decomposition of protein to formation of nitrite (Fig. 6). Plants normally use N in the forms of ammonium (NH_4^+) and/or nitrate (NO_3^-) [30]. Nitrification (conversion of NH_4^+ to NO_3^-), which is one of the most important reactions of nitrogen cycle in agricultural soils, can be used to evaluate the N circulation activity [31]. To evaluate the N circulation activity, a new method was developed here. Upland agricultural soil samples were evaluated for N circulation activity using this method.

Fig. 4. Agarose gel electrophoresis of eDNA extracted from soil using various eDNA extraction methods. Lane 1, the slow-stirring method; lane 2, the heat treatment method and lane 3, the bead beating method.

A new method was developed to estimate the N circulation activity of soils in our previous study [31]. In this method, the activity is analyzed based on the bacterial biomass, ammonium oxidation rate, and nitrite oxidation rate in the soil. The bacterial biomass in the soil is analyzed by the eDNA quantification method [29]. To estimate the rates of ammonium and nitrite oxidation in the soil sample, water-holding capacity of the soil sample is adjusted to 60%. Then ammonium sulfate or sodium nitrite is added to the soil (60 μg N g^{-1} dry soil) sample. Soil sample without the addition of ammonium sulfate or sodium nitrite is used as a control experiment. After 3 days of incubation at 25 °C, the percentage of reduction in the added N is defined as the ammonium or nitrite oxidation rates. The bacterial

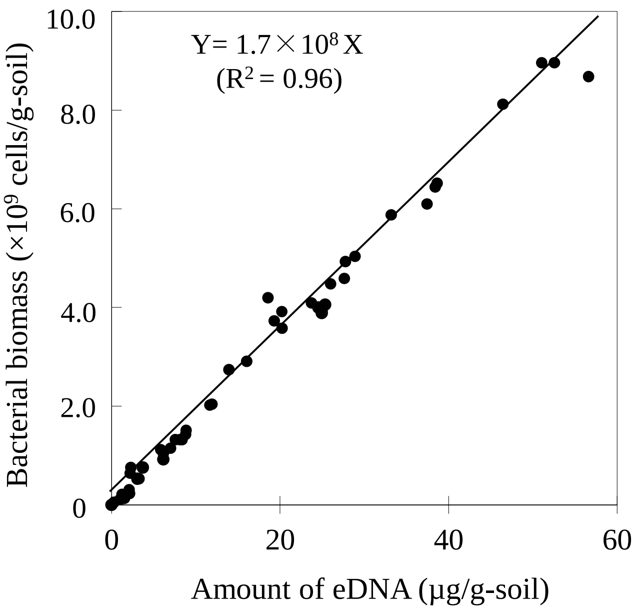


Fig. 5. Relationship between the bacterial number obtained by DAPI staining method and the amount of eDNA extracted by the slow-stirring method (n = 57).

biomass of 6.0 × 10^8 cells g^{-1} and 2.0 × 10^8 cells g^{-1} soil are defined as 100 points and 0 point, respectively. Using the scores of bacterial biomass, ammonium oxidation rate, and nitrite oxidation rate, a radar chart is constructed, and the relative area of the inner triangle is expressed as the N circulation activity (Fig. 7).

The area of the inner triangle in the radar chart is calculated as follows:

$$\text{Area} = \frac{(a\times b) + (b\times c) + (c\times a)}{4} \times \frac{\sqrt{3}}{100}$$

where a, b, and c denote scores of bacterial number, ammonium oxidation rate, and nitrite oxidation rate, respectively.

Nitrogen circulation activity is analyzed by calculating the relative area of inner triangle as follows:

$$\text{N circulation activity (point)} = \frac{\text{Area of the inner triangle}}{\text{Area of the outer triangle}} \times 100$$

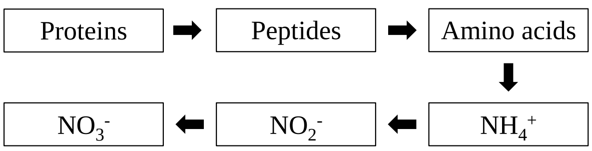


Fig. 6. N circulation activity showing major steps of conversion of organic N (protein) to inorganic N.

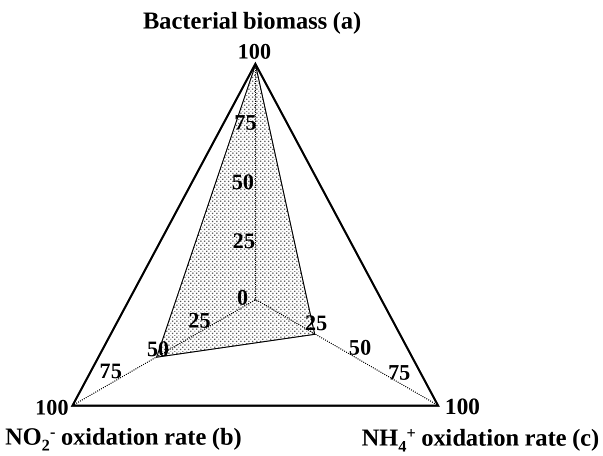


Fig. 7. Radar chart used to calculate N circulation activity.

2.2.2. Phosphorus circulation activity

Phosphorus circulation is also one of the most important material circulations in the soil environment. Microbes mediate the conversion of unavailable forms of inorganic and organic P to available forms such as $H_2PO_4^-$ and HPO_4^{2-} [32-34]. Organic P constitutes a large fraction of total P in the agricultural soil [34-36]. However, chemical fixation of soluble P in the forms of phosphates of iron and aluminum is a big challenge (Fig. 8).

A new method for the evaluation of P circulating activity was developed in our previous study [34]. This method is based on the soluble P formation from organic P in soil (Fig. 9). In this method, phytate (the most dominant form of soil organic P) is used as a substrate. At first, phytate solution (pH 7.0) containing 3.9 mg of P is added to 1.0 g of soil sample and is incubated for 3 days at room temperature at 60% water holding capacity. Control experiment (without phytate) is carried out simultaneously. Soluble phosphorus (SP) is extracted from the incubated 1.0 g soil sample with 20 mL distilled water, and is analyzed by the molybdenum blue method [37]. The increment in SP in phytate added soil after 3 days is defined as the P circulation activity in the soil. The circulation activity is expressed in points assigning 0 point for no circulation activity and 100 points for full circulation activity of the added phytate P.

3. RESULTS AND DISCUSSION

3.1. Analysis of bacterial biomass in agricultural soils

A total of 235 samples collected from upland agricultural soils were analyzed for bacterial biomass using eDNA quantification method (Fig. 10). The bacterial biomass ranged between 1.0 × 10^7 and 2.6 × 10^9 cells/g-soil with an average of 6.0 × 10^8 cells/g-soil. Up to 100 times difference in bacterial biomass among the soil samples might be due to the variation in soil texture, cropping system, fertilizer use, season, pH, etc. Although, the bacterial biomass is a good biological indicator of soil fertility, analysis of specific microbial functions, such as material circulation activity, is also necessary for evaluating soil fertility.

3.2. Analysis of N circulation activity

N circulation activities of upland agricultural soils (235 samples) were analyzed (Fig. 11). Average

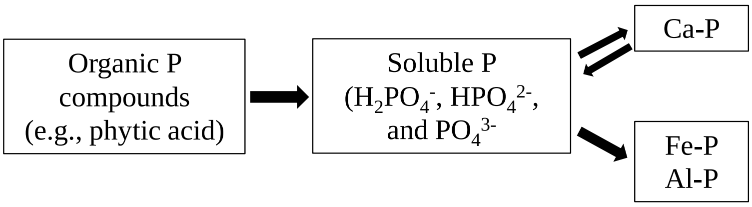
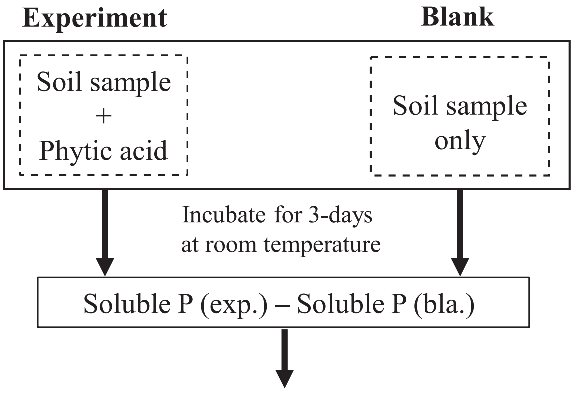


Fig. 8. P circulation activity showing conversion of organic P to inorganic forms in the agricultural soil environment.



$$\text{P circulation activity (point)} = \frac{\text{Soluble P (exp.)} - \text{Soluble P (bla.)}}{\text{Added phytic acid P}} \times 100$$

Fig. 9. Procedure for estimating P circulation activity of soil.

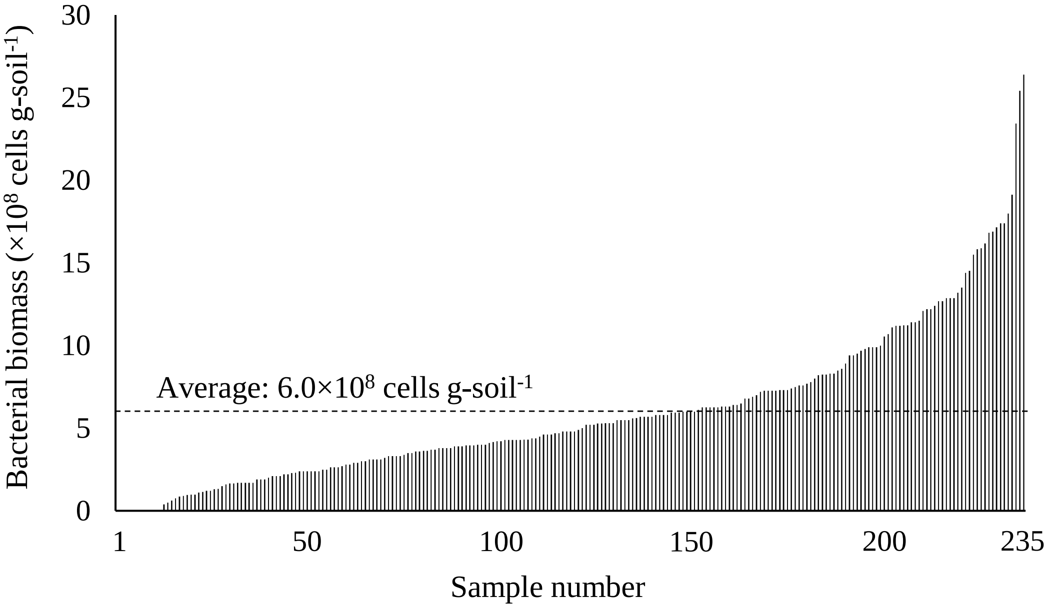


Fig. 10. Bacterial biomass in upland agricultural soils (number of samples = 235).

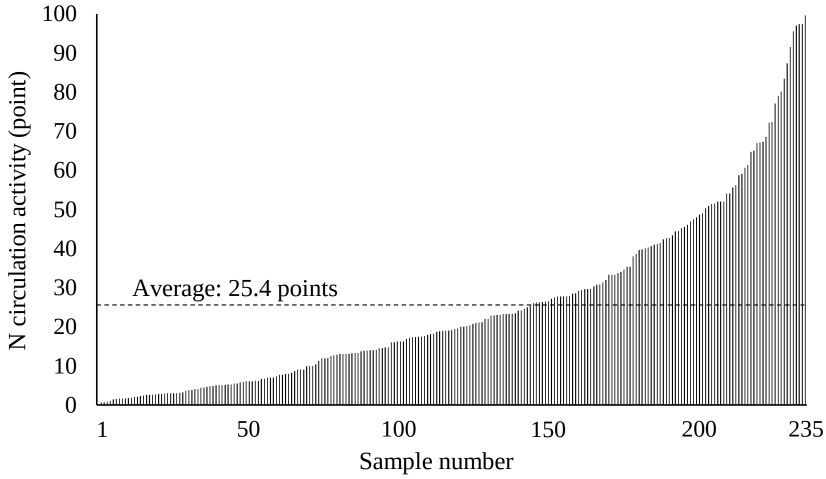


Fig. 11. Nitrogen circulation activity in upland agricultural soils (number of samples = 235).

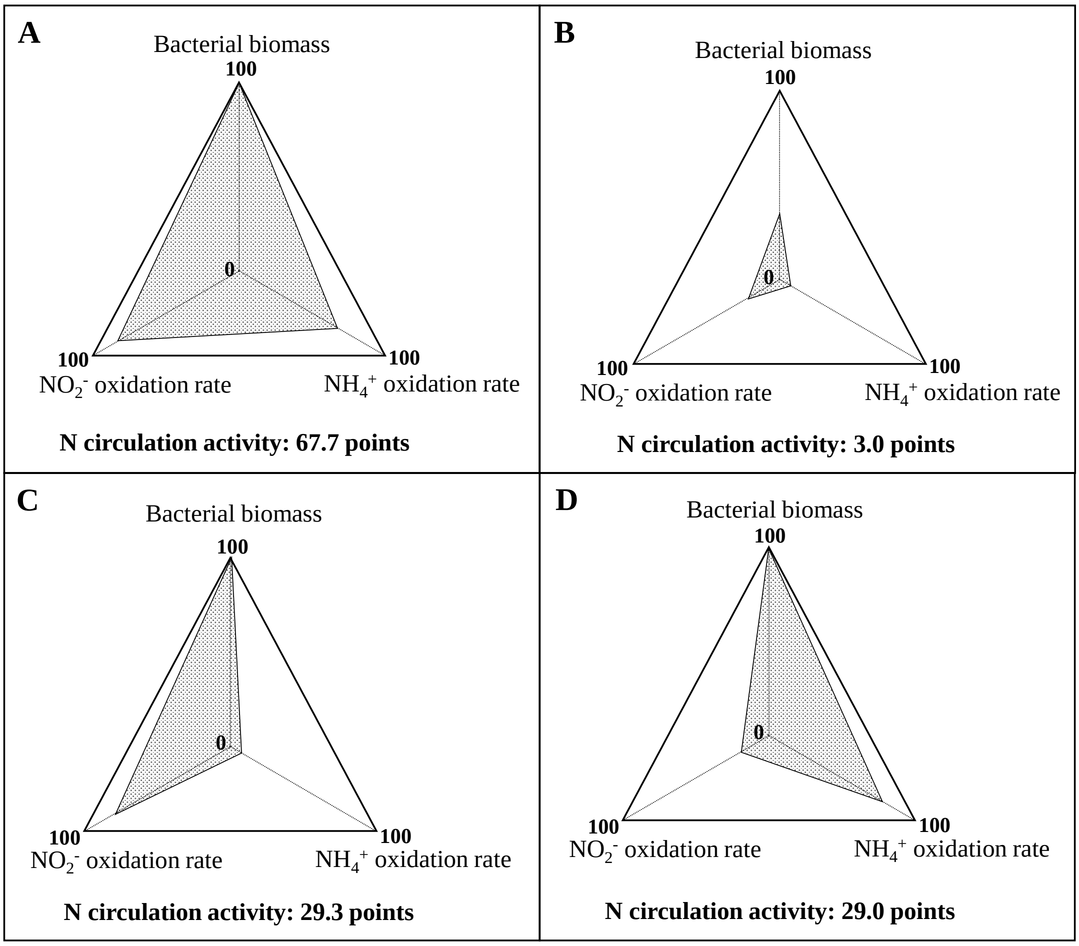


Fig. 12. Radar charts showing different patterns of N circulation activities in representative soil samples. A: high level of N circulation activity; B: low level of N circulation activity; C: low level of ammonium oxidation activity and D: low level of nitrite oxidation activity.

N circulation activity was 25.4 points and the activities were quite different among the samples. Four major patterns of N circulation activity are shown in Fig. 12.

3.3. Analysis of P circulation activity in upland agricultural soils

P circulation activity of the same soil samples (235 samples) were analyzed (Fig. 13). The average P circulation activity was 32.1 points. High variations in P circulation activity (0 to 100 points) were observed among the samples, suggesting that microbial biomass and mineral contents such as Ca, Fe, Al, etc. were different in each agricultural soil.

3.4. Analysis of suitable conditions for enhancing bacterial biomass and N circulation activities in soil

A large number of soil samples (146 out of 235) had carbon-to-nitrogen ratio (C/N ratio) within the range of 8-25 (Fig. 14). Optimal conditions of total

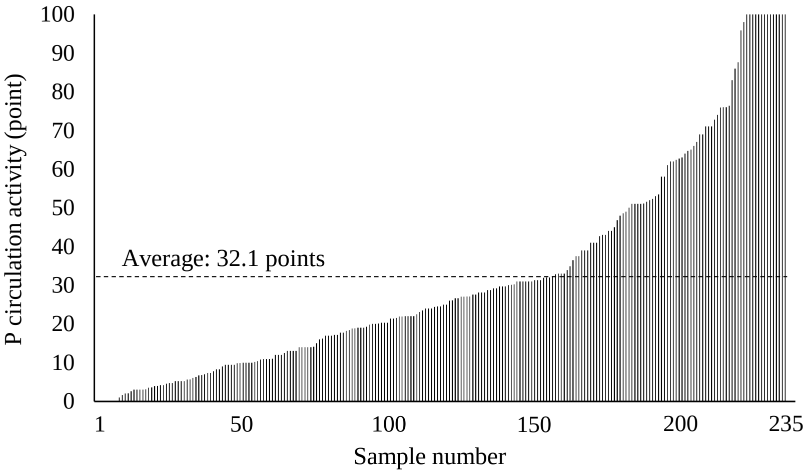


Fig. 13. P circulation activity in upland agricultural soils (number of samples = 235).

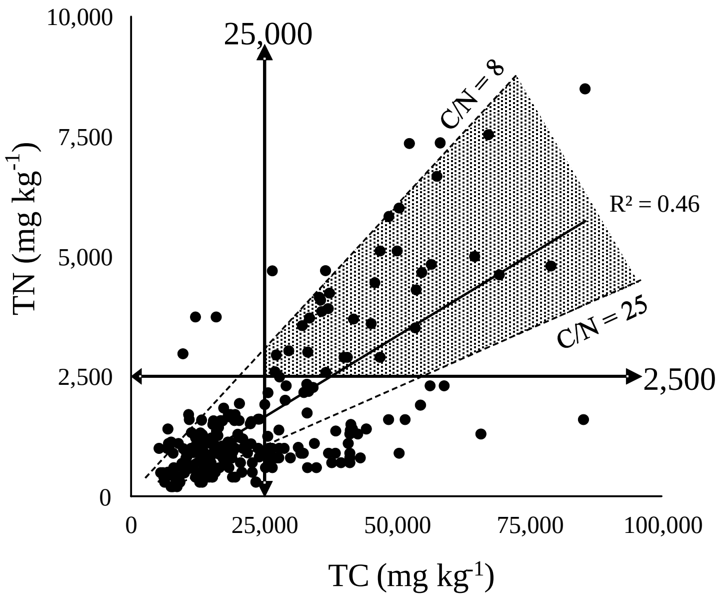


Fig. 14. Relationship between TC and TN in the 235 upland agricultural soils. Area under shadow indicates the most suitable TC, TN, and C/N ratio for enhancing N circulation activity.

Table 1. Analysis of suitable TC and TN conditions for bacterial biomass and N circulation activities.

C/N	TC (mg/kg)	TN (mg/kg)	Nitrification activity (point)	Bacterial biomass (× 10^8 cells/g)	NH_4^+ oxidation rate (point)	NO_2^- oxidation rate (point)	Number of samples
8-25	≥ 10,000	≥ 1,000	29.3	6.4	35.1	58.8	97
	≥ 15,000	≥ 1,500	31.3	6.5	37.9	61.7	61
	≥ 20,000	≥ 2,000	36.4	6.7	42.2	68.8	43
	≥ 25,000	≥ 2,500	37.7	6.5	41.0	70.0	34
	≥ 30,000	≥ 3,000	43.3	7.3	45.8	75.8	27
	≥ 40,000	≥ 4,000	47.5	8.2	49.3	85.1	15
	≥ 50,000	≥ 5,000	53.9	8.7	56.1	87.9	7

Soil fertility index (SOFIX) report card

Sample name: Potato Field No. 2

Property	**Value**	**Optimum value**	**Evaluation**
NH_4^+-N	25	50 to 200	↓
NO_3^--N (mg/kg)	0	50 to 200	↓
Soluble P_2O_5 (mg/kg)	210	50 to 200	↑
Soluble K_2O (mg/kg)	109	50 to 200	○
C/N ratio	31	8 to 25	↓
C/P_2O_5 ratio	11	8 to 15	○
TC (mg/kg)	35,400	≥ 25,000	○
TN (mg/kg)	1,200	≥ 2,500	↓
TP_2O_5 (mg/kg)	3,300	≥ 3,000	○
TK_2O (mg/kg)	5,500	≥ 3,000	○
N circulation activity (pt.)	35.0	≥ 50	↓
Bacterial biomass (× 10^8 cells/g)	5.2	≥ 6.0	○
NH_4^- oxidation rate (pt.)	10	≥ 60	↓
NO_2^- oxidation rate (pt.)	100	≥ 60	○
P circulation activity (pt.)	100	≥ 30	○
pH	7.2	5.5 to 6.5	↑
EC (dS/m)	0.3	0.2 to 1.2	○

Fig. 15. An example of evaluating soil fertility by SOFIX. Optimum values are the amounts needed for an efficient organic agriculture. Upward arrow, circle, and downward arrow indicate higher, optimum, and lower than the optimum values, respectively.

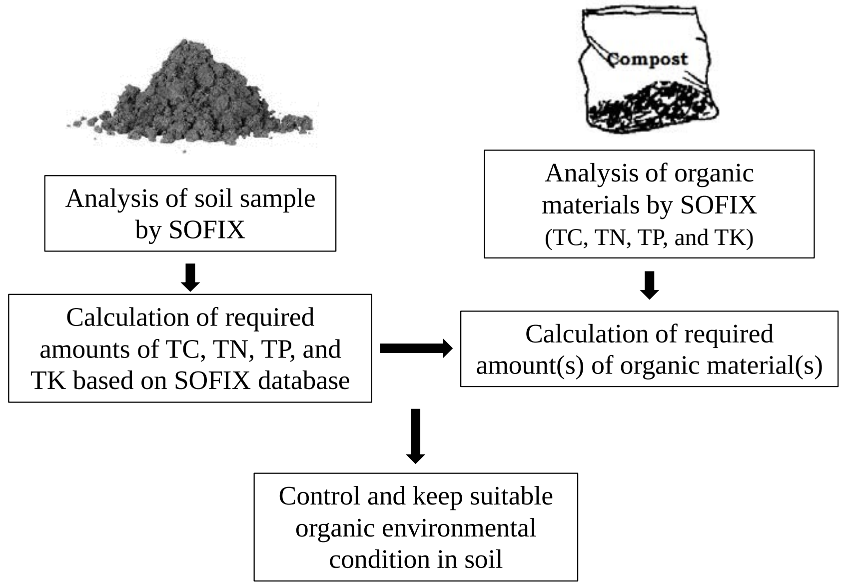


Fig. 16. Procedure for soil preparation based on SOFIX.

carbon (TC) and total nitrogen (TN) for enhancement of N circulation were investigated within the C/N range of 8-25.

N circulation activity increased when the levels of TC and TN were high. N circulation activity at TC $\geq$ 50,000 mg/kg and TN $\geq$ 5,000 mg/kg reached 53.9 points, which was 1.8 times higher than that at TC $\geq$ 10,000 mg/kg and TN $\geq$ 1,000 mg/kg (Table 1). Based on the N circulation activities and the corresponding number of samples at various levels of TC and TN, the required conditions for enhancing the activity in upland soils were as follows: C/N ratio 8-25, TC $\geq$ 25,000 mg/kg, and TN $\geq$ 2,500 mg/kg. The results indicate that N circulation activity is influenced by TC, TN, and the C/N ratio in the upland soil environment.

4. CONCLUSION

Bacterial biomass, N circulation activity, and P circulation activity were closely related to TC, TN, and total phosphorus (TP). Enhancement of these parameters will contribute to an efficient organic agricultural system, which is a logical conclusion. For an efficient organic material-based agriculture, SOFIX-based organic material treatments is proposed. Suitable conditions (TC, TN, TP, C/N ratio, etc.) for higher levels of bacterial biomass and nutrient circulation activities are determined. The suitable conditions are C/N ratio 8-25, TC $\geq$ 25,000 mg/kg, TN $\geq$ 2,500 mg/kg and TP $\geq$ 3,000. Values to be improved for an efficient organic material-based agriculture system can be easily recognized from the data of SOFIX (Fig. 15).

The proposed procedure for improvement of fertility of agricultural soil with organic materials based on SOFIX is illustrated in Fig. 16.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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