Influence of aerobic treated manure application on the chemical and microbiological properties of soil

Adriana Montañez (Montañez, A)1, Natalia Rigamonti (Rigamonti, N)1, Silvana Vico (Vico, S)1, Carla Silva (Silva, C)1, Lucía Nuñez (Nuñez, L)1 and Stella Zerbino (Zerbino, S)2

1Universidad de la República Oriental del Uruguay, Facultad de Ciencias, Instituto de Ecología y Ciencias Ambientales, Laboratorio de Microbiología de Suelos. Iguá 4225, 11400 Montevideo, Uruguay.
2INIA. La Estanzuela, 70000 Colonia, Uruguay.

Abstract

Aim of study: This study evaluated the effect of the application of liquid aerobic treated manure (continuous liquid composting, CLC) on physical, chemical and biological soil properties, with the objective of monitoring changes induced by soil management with CLC as a biofertilizer.

Area of study: Colonia, Uruguay (lat. 34,338164 S, long. 57,222630 W).

Material and methods: Soil’s chemical properties, including nitrogen mineralization potential (NMP) and 15 microbiological properties (microbial biomass carbon, MBC; mesophylic aerobic bacteria; actinobacteria; filamentous fungi; fluorescein diacetate hydrolysis; dehydrogenase; with NMP; acid and alkaline phosphatase; cellulolose degraders; P-solubilizing bacteria; nitrifying; denitrifying and free-living N-fixing microorganisms; glomalin; and soil-pathogenicity index, SPI) were evaluated in two sites with similar cropping history, with one and three years of respective CLC application.

Main results: CLC application had significant effects on soil microbial biomass (p<0.05), soil enzyme (p<0.1) and functional groups activity (p<0.05). SPI decreased in both sites with CLC application. No significant variations were detected for the chemical variables, with the exception of NMP, which was significantly high (p<0.05) in soil treated with CLC at both sites.

Research highlights: The improved biological soil properties analyzed (MBC, soil enzyme activities and SPI, together with NMP) emerged as reasonable indicators to assess and monitor the effects of CLC application.

Additional keywords: organic fertilization; microbiological indicators.

Introduction

In order to achieve a sustainable agriculture, it is necessary to reduce the need for mineral fertilization input. The FAO Voluntary Guidelines on Sustainable Soil Management (VGSSM) encourage the adoption of agricultural practices that build and retain soil organic matter (FAO, 2017). The total or partial replacement of mineral fertilizer with organic amendments is a good practice to solve the problems of the excessive use of mineral fertilizer while improving physical and chemical soil properties, carbon stocks and soil biodiversity (Gattinger et al., 2012; Sradnick et al., 2013). The application of manure and compost on
agricultural lands has shown a positive increase in water and nutrient retention, nutrient cycling, carbon transformation, soil biodiversity, soil structure and soil aggregation while enhancing soil organic matter content (Treonis \textit{et al.}, 2010; Nair & Ngouajio, 2012) and suppressing soil-borne pathogens (Zaccardelli \textit{et al.}, 2013). The supply of manure to agricultural soils is an ancient practice and a well-tested strategy to increase soil organic matter (SOM), replenish basic plant nutrients, improve yield response to fertilizers and restore soil productivity in degraded areas (Schröder, 2005; Rufino \textit{et al.}, 2007; Bogaard \textit{et al.}, 2013; Nezomba \textit{et al.}, 2015). At the same time, excessive manure applications and inefficient manure storage practices can have detrimental effects on the environment at multiple scales, such as the contamination of water and soil resources at local and regional levels, and the increase of greenhouse gases emissions (GHG) at a global level (Sutton, 2011; Tubiello \textit{et al.}, 2013). Manure is rich in nitrogen, phosphorus and carbon (Pagliari & Laboski 2012) and can contribute to increase global climate change through the emission of methane and nitrous oxide (Leytem \textit{et al.}, 2011). Thus, while the recycling of livestock manure within agricultural systems is necessary to improve and maintain soil health, its efficient management is also important to reduce the environmental impact of farming activities. Therefore, manure treatment becomes a focal issue in relation to current national policies on environmental, climate and renewable energy matters.

Uruguayan agriculture has intensified during the last 15 years, with a large input of mineral fertilizer and other agrochemicals. In recent years, the application of manure has attracted increased attention, and nowadays a group of Uruguayan farmers is incorporating a new technology based on an \textit{in situ} aerobic process of the manure treatment that consists of continuous liquid composting (CLC) in aerated tanks. There are 52 tanks installed in the country, and the liquid manure composting is applied throughout ~ 8,000 ha. The assessment of soil quality after CLC application is essential to track changes in soils as a result of management practices. Within the framework of sustainable agricultural production, a high soil quality and health should maintain a high productivity without significant soil or environmental degradation (Govarts \textit{et al.}, 2006; Griffiths \textit{et al.}, 2010; Bünemann \textit{et al.}, 2018). A set of biological soil indicators must be selected in order to advise farmers as to whether this management strategy yields the positive changes they anticipated.

Soil biological indicators are considered good soil quality indicators due to their sensitivity and ability to reflect soil management effects (McGuire & Treseder, 2010; Bowles \textit{et al.}, 2014; Benintende \textit{et al.}, 2015). Generally, soil health has been related to the soil organic matter content (Gao, 2006), soil fertility and structure, total N and C/N ratio (Murphy \textit{et al.}, 2011), but these soil properties do not necessarily change as a result of changing external conditions or use (Chodak & Niklińska, 2010; Preem \textit{et al.}, 2012; Muscolo \textit{et al.}, 2014), and hardly reflect short-term changes in soil processes associated to new environmental threats. Changes in soil characteristics or environmental conditions induce rapid changes on microbial biomass, community composition and activity (Schloter \textit{et al.}, 2003; Gil-Sotres \textit{et al.}, 2005; Nogueira \textit{et al.}, 2006; Qin \textit{et al.}, 2010). In some cases, changes in microbial communities and activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation (Muscolo \textit{et al.}, 2015).

However, there is still a lack of consensus on what to measure for the evaluation of soil biota linked to soil health and the ensuing prediction of sustainability or productivity (Saha \textit{et al.}, 2008; Dong \textit{et al.}, 2014). Most of the studies about biological soil quality indicators propose measuring key functional microbial groups, as well as specific biological properties such as soil enzyme, microbial biomass carbon or N, basal respiration, FAME profile, genomic analysis, and glomalin concentration (Huang \textit{et al.}, 2014; Dose \textit{et al.}, 2015). These parameters can be used for the evaluation of soil health and as early indicators of soil degradation. However, the characterization of multiple microbiological soil parameters to enhance the understanding of the correlation between soil biota and positive or negative effects on microbial functions and ecosystem services has not been as widely regarded.

The objectives of this study were: (i) to evaluate the influence of aerobically treated liquid manure (CLC) on soil microbial properties on two different agricultural sites with short- and medium-term CLC application (1 and 3 years, respectively), and (ii) to identify a minimal data set of microbiological parameters to monitor early changes induced by soil management with CLC as a biofertilizer.

\section*{Material and methods}

\subsection*{Biofertilizer: liquid manure composting}

The biofertilizer used in the experiment was obtained through the \textit{in situ} aerobic treatment of livestock manure according to the method developed at the University of São Paulo, Brazil (D’Andrea & Medeiros, 2002). In brief, the liquid biofertilizer was obtained by a continuous system of aerobic biodegradation in aerated
Influence of manure application on the chemical and microbiological properties of soil

The studied area was located in Colonia, Uruguay (lat. 34.338164 S, long. 57.222630 W). The climate of the area is classified as humid subtropical, with an average temperature of 24 °C and an annual mean precipitation of 1200 mm. The soil is an Eutric Cambisol (FAO, 1998) or Brunosol Eutrico according to Durán’s soil classification (Durán, 1991). Within this area, two adjacent sites were selected with one year (S1y) and three years (S3y) of CLC application and a similar crop sequence (Table 1). At each site, the experiment had a completely randomized block design with four replicate plots (144 m² each) of two treatments: CLC amendment (CLC) and no CLC application (NCLC) plots. CLC was applied in two doses post crop sowing, half in September, after the summer crop emergence (2-3 leaves), and the other half in autumn, after the winter crop emergence, completing a total rate of 300 L ha⁻¹ year⁻¹. Within each block, the distance between plots was 12 m. All treatments received mineral fertilization. The mineral fertilization in both sites during maize cultivation was: 50 kg ha⁻¹ of P, 50 kg ha⁻¹ of N in the form of urea (50% at maize seeding and 50% at the 4th leaf stage), and 50 kg ha⁻¹ of KCl.

Table 1. Crop sequence and application of continuous liquid composting (CLC) before spring 2015 in the two sites selected for this study: S1y (site with one year of CLC application) and S3y (site with three years of CLC application). NCLC: no application.

| Year/Season | S3y          | S1y          |
|-------------|--------------|--------------|
| 2013 Autumn | Cover crop / CLC | Cover crop / NCLC |
| 2013 Spring | Sorghum / CLC  | Sorghum / NCLC  |
| 2014 Autumn | Wheat / CLC   | Wheat / NCLC   |
| 2014 Spring | Soybean / CLC | Soybean / CLC |
| 2015 Autumn | Cover crop / CLC | Wheat / CLC    |
| 2015 Spring | Maize / CLC   | Maize / CLC   |

Soil sampling

Soil samples were collected from the two sites in September 2015, after the maize harvest. Composite soil samples from eight plots of 144 m² were collected from each site, four with CLC application and four without it (NCLC). Composite soil samples consisted of 10 soil cores (20 cm depth, 10 cm diameter) from each plot. Each composite soil sample was well homogenized in sterile plastic bags and divided into two parts, for chemical and microbial analysis. Soil samples for microbial analysis were sieved through a 2 mm mesh and stored at -20°C for microbial biomass and enzyme analyses, and at 4°C for culturable biodiversity and functional groups assessment. Soil samples for chemical analysis were sieved through a 2 mm mesh and air dried.

Physicochemical analysis of soil

The pH of a 1:2.5 (w/v) soil/water suspension and the total organic carbon (SOC) (Walkley & Black, 1934), available PO₄³⁻ (Bray & Kurtz, 1945), inorganic N (N-NO₃⁻) (Goldman & Beegle, 1998) and exchangeable K (Toth & Prince, 1949) in the soil were determined. The soil’s water content was measured after drying soil at 105°C for 24 h. The N-mineralization potential (NMP) was determined according to Waring & Bremner (1964). In brief, 20 g of soil were incubated under waterlogged conditions for 7 days at 25 °C in stoppered 50 mL tubes. After the incubation period, N in the resulting slurry was extracted with a 4 M KCl solution (1:10 extraction ratio). Ammonia was distilled, trapped in boric acid solution (2%) and titrated with sulfuric acid (0.005 N). The NMP was determined by subtracting the initial NH₄⁺ content in the soil samples from the concentration of NH₄⁺ determined after the incubation (Waring & Bremner, 1964).

Biochemical and microbiological properties

Soil microbial biomass

Microbial biomass carbon (MBC) was determined by a chloroform-fumigation extraction method (Vance et al., 1987). An extraction efficiency coefficient of 0.35 was used to convert soluble C into biomass C (Spa- larling et al., 1990).

Culturable mesophilic aerobic microorganisms

Total mesophilic aerobic bacteria (MAB), filamentous fungi (MFF) and actinobacteria (MAA) were evaluated by means of a plate counting technique. Plates were incubated at 28°C and the results were expressed as colony-forming units (cfu) per gram of dry soil. MAB were enumerated on TY (Beringer, 1974) with 1 mL amphotericin B (1.5 mg mL⁻¹); MFF, on rose bengal agar with 1 mL of streptomycin (3 mg mL⁻¹) (Frioni, 2011) and MAA, on actinomycetes isolation
agar (Frioni, 2011). MAB were enumerated at 48 h of incubation, whereas MFF and MAA, after 72 h.

Microbial functional groups related to soil fertility

The populations of microbial functional groups involved in carbon (soil-borne cellulose degraders: cellulolytic population), phosphorus (P-solubilizing microorganisms) and N (free living N-fixing, ammonium-oxidizing and denitrifying microorganisms) were analyzed. The quantification of the cellulolytic population was determined by a most-probable-number (MPN) count technique. Tubes with minimal medium with a 50 mg piece of Whatman 1 paper as the sole C source were incubated at 28°C, 140 rpm for 20 days (Frioni, 2011). For P solubilizing microorganisms, tenfold dilutions were spread on duplicate plates containing National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999). To yield 1:10 soil dilution, 10 g of soil was added to 90 mL of sterile phosphate buffer (10 mM, pH =7) with two drops of 2.5% Tween and agitated at 15 min at 200 rpm. Serial dilutions were produced by taking 1 mL (1:10 dilution) in 9 mL of phosphate buffer. Plates were incubated at 28°C and the results were expressed as CFU g⁻¹ of dry soil. P solubilizing activity was considered if a solubilization halo was present on the respective culture medium. The MPN technique was used for quantifying the nitrifying, denitrifying and free-living N-fixing microaerophilic organisms (Schmidt & Belser, 1994; Schinner et al., 1996).

Soil enzyme activity

Enzymatic activities of fluorescein diacetate hydrolysis (FDA), dehydrogenase (DHA), and alkaline and acid phosphatase enzymes (AlPh and AcPh) were assayed based on the colorimetric determination of the product released by the enzyme. Enzyme activities were expressed as micrograms of product per gram of dry soil per specified time. FDA hydrolysis reaction was determined according to the methods of Adam & Duncan (2001); DHA activity, according to the procedure of Casida et al. (1964). AlPh and AcPh enzymes were determined with p-nitrophenyl phosphate by the methodology described by Eivazi & Tabatabai (1977).

Glomalin

The extraction of glomalin-related soil protein (GRSP) was performed by adding 8 mL of 50 mM trisodium citrate dehydrate solution at pH 8 in a centrifuge tube and then autoclaving it at 121°C for 60 min. After each extraction, the sample was centrifuged at 3220 rpm for 15 min and the supernatant containing glomalin was collected and stored at 4°C. At least four sequential extractions were carried out until the supernatant showed yellow pale color indicating the absence of glomalin. GRSP was then quantified by means of the Bradford dye-binding assay (Wright & Upadhyaya, 1998).

Soil suppressive capacity

The soil pathogenicity index (SPI) is an indicator of soil suppressive capacity. Ten surface-sterilized soybean seeds were placed on 40 mL soil and 10 mL sterile water placed on paper tissue and rolled. Each pack was placed in a sterile plastic bag. Sterile and unsterile soil was evaluated, counting the number of seeds that germinated and emerged in the dark after 10 days at 22 °C. The SPI was calculated as follows: SPI = (N° of seeds emerged in sterile soil – N° of seeds emerged in unsterile soil) / N° of seeds emerged in sterile soil (Altier & Zerbino, 2012). SPI values vary from zero to one, zero being no soil pathogenicity.

Statistical analysis

The sites were treated as independent experiments, and the main treatment for statistical comparison was presence (CLC) and absence (NCLC) of CLC application. All data were tested for normality using the Shapiro-Wilk test and variance homogeneity was assessed by means of the Levene test. Data of celluloses degraders, FDA, N-fixation, and NH₄⁺ oxidizers were log transformed to meet the normality assumption of the statistical tests. The effect of CLC application on soil microbiological and chemical properties was analyzed using a factorial analysis of variance (ANOVA). Fisher’s LSD was used to identify significant differences (p<0.05 or p<0.1). The relationship between variables was determined by multiple correlation analysis. The correlation analysis between soil pH, moisture and microbiological parameters was carried out using Spearman’s correlation coefficient. Principal Component Analyses (PCA) were performed to analyze the ordination of the treatments according to physicochemical and biology soil properties. Statistical analyses were performed using the software InfoStat (Di Rienzo et al., 2016) with the interface software R (http://www.r-project.org/).

Results

Fifteen different microbial variables related to several soil biological functions and seven physicochemical parameters were analyzed (Table 2). The study showed variations in most of the evaluated microbiological properties of treated and untreated soil in each site
Influence of manure application on the chemical and microbiological properties of soil

Table 2. Mean values (n=4) and standard deviation of soil physicochemical parameters for each site biofertilized with continuous liquid composting (CLC) and not biofertilized (NCLC).

| Physicochemical parameters | S3y NCLC | S3y CLC | S1y NCLC | S1y CLC |
|-----------------------------|---------|---------|----------|---------|
| pH                          | 5.5 (0.15) | 5.4 (0.01) | 5.7 (0.17) | 5.6 (0.05) |
| Humidity (%)                | 19.0 (1.88) | 18.8 (1.42) | 18.9 (1.92) | 20.5 (1.29) |
| SOC (%)                     | 5.3 (0.37) | 4.5 (0.21) | 2.5 (0.51) | 2.5 (0.11) |
| N-NO₃ (µg N g⁻¹)            | 10.6 (3.54) | 12.2 (3.15) | 12.2 (4.72) | 10.8 (3.26) |
| P (µg P g⁻¹)                | 45 (4.20) | 54 (5.77) | 10.8 (3.86) | 9.3 (4.11) |
| K (mg 100 g⁻¹)              | 0.6 (0.21) | 0.9 (0.08) | 0.4 (0.03) | 0.4 (0.03) |
| NMP (µg N g⁻¹)              | 25* (0.99) | 33* (0.14) | 32 * (6.55) | 52* (0.11) |

SOC: total soil organic carbon. N-NO₃: inorganic nitrogen. P: soil available PO₄. K: exchangeable potassium. NMP: N-mineralization potential. S3y: site with three years of CLC application. S1y: site with one year of CLC application. Significance differences recorded among NCLC and CLC treatments at each site according to the LSD test (*p < 0.05).

(S1y and S3y, with one and three years of CLC application, respectively) (Table 3). S1y and S3y are adjacent sites with Eutric Cambisols. According to the physicochemical parameters analysed (Table 2), the two sites showed differences in SOC% and P content before the application of CLC. To avoid site effect, the main treatment for statistical comparison was presence (CLC) and absence (NCLC) of CLC application within each site. The statistical analysis of the main soil physicochemical properties within each site did not show changes with CLC application (Table 2). However, NMP was statistically higher (p<0.05) in CLC amended plots at both sites.

MBC increased with the application of CLC in both sites. The increase was 13.0% in S3y and 23.3% in S1y; the increase was statistically significant only in the latter case (Table 3). The application of CLC had positive and negative significant effects on the mesophilic aerobic culturable microorganisms. Increases in cfu g⁻¹ of MAB at S1y (p<0.1) and MAA at S3y were recorded, as well as a decrease in MFF at S3y (p<0.05, Table 3). These results indicate that the effects of the application of CLC on the recorded increases or decreases in the mesophilic culturable population were not consistent.

In both sites (S1y and S3y), there was a significant increase in the MPN of the functional groups as a result of the application of CLC (Table 3). In both sites, the application of CLC caused an increase in the values of cellulolytic and diazotrophic microorganisms. However, the CLC and NCLC treatments were only statistically different in S3y (p<0.05). The density of P solubilizing bacteria was lower in CLC treatment in both sites, but the differences were only significant in S3y (p<0.1). MPN of denitrifying microorganisms increased with CLC application in both sites (Table 3). In addition, we recorded significant positive correlations between MFF and P solubilizing microorganisms; MAB and NH₃ oxidizers; MAA, N-fixing microorganisms and denitrifiers (Table 4).

The activities of four enzymes (FDA, DHA, AlPh, AcPh) increased in both sites with the application of CLC (Table 3). However, the changes in FDA and DHA activities were not statistically significant. AcPh activity increased significantly in CLC treated plots at both sites (S1y, p<0.05; S3y, p=0.1).

In the case of glomalin, contrary to what was expected, our results did not show a significant change in GRSP with CLC treatment in neither site (Table 3). Glomalin was positively correlated with MAA and negatively correlated with enzyme activities (p<0.05, Table 4).

SPI decreased with CLC treatment, although the differences were only significant in S3y (p<0.05, Table 3). Therefore, a medium-term application of CLC (3 years) significantly increased the suppressing capacity of soil pathogens. Positive and negative significant correlations were found between SPI, enzymes and some functional groups (Table 4).

The results of the PCA carried out with physicochemical and biological soil properties indicate that the two first components (PC1 and PC2) accounted for 88.3% of the total data variation (Fig. 1). The first axis opposed sites S1y and S3y (61.5% of variance explained). The biological soil properties DHA, AcPh and FDA, associated with S1y, and denitrifying, SPI, MAA and glomalin, associated with S3y, were important for the ordination of treatments in PC1. The second axis grouped CLC and NCLC treatments at each site (26.8% of variance explained). On the other hand, P solubilizers and MFF, associated with NCLC,
Table 3. Mean values (n=4) of the selected microbiological variables measured in plots biofertilized and not biofertilized with CLC (CLC and NCLC, respectively).

| MICROBIAL PARAMETERS | S3y NCLC | S3y CLC | S1y NCLC | S1y CLC |
|----------------------|---------|---------|---------|---------|
| MBC (μg C 100-g⁻¹)  | 244.26 (123.77) | 279.12 (62.10) | 69.45 ** (22.92) | 90.56 ** (4.03) |
| Mesophilic aerobic microorganism (cfu g⁻¹ dry soil) | MAB | 4.7 · 10⁶ (0.47) | 4.4 · 10⁶ (0.78) | 5.5 · 10⁶ ** (0.54) | 7.7 · 10⁶ ** (1.60) |
| | MAA | 1.9 · 10⁸ * (0.30) | 2.4 · 10⁸ * (0.18) | 1.5 · 10⁸ (0.69) | 1.2 · 10⁸ (0.67) |
| | MFF | 19.84 · 10⁶ * (5.02) | 9.2 · 10⁶ * (0.38) | 1.0 · 10⁶ (1.2) | 2.0 · 10⁶ (1.4) |
| Functional groups | Cellulose degraders (MPN g⁻¹ dry soil) | 1.8 · 10⁸ * (1.4) | 23.2 · 10⁸ * (9.9) | 5.5 · 10⁸ * (0.7) | 15.6 · 10⁸ * (0.06) |
| | P-solubilizing (cfu g⁻¹ dry soil) | 10.6 · 10⁶ ** (6.6) | 4.1 · 10⁸ ** (0.6) | 7.3 · 10⁸ (0.9) | 7.0 · 10⁸ (4.3) |
| | N-fixation (MPN g⁻¹ dry soil) | 9.2 · 10⁶ * (7.2) | 39.6 · 10⁶ ** (19.3) | 7.7 · 10⁶ (6.3) | 35.9 · 10⁶ (34.7) |
| | NH₄⁺ oxidizers (MPN g⁻¹ dry soil) | 3.86 · 10⁸ ** (1.4) | 1.03 · 10⁸ ** (0.1) | 3.87 · 10⁸ (1.8) | 6.3 · 10⁸ (3.4) |
| | Denitrifying microorganism (MPN g⁻¹ dry soil) | 6.3 (2.0) | 12.5 (6.9) | 0.4 (0.02) | 0.73 (0.3) |
| Soil enzymes activity | FDA (μg FDA g⁻¹,0.5-h⁻¹) | 9.17 (0.74) | 9.52 (0.84) | 42.43 (36.94) | 53 (47.79) |
| | DHA (μg INTF g⁻¹ 2-h⁻¹) | 29.81 (10.76) | 40.31 (11.86) | 196.72 (43.56) | 208.74 (28.20) |
| | AlPh (μg pNP g⁻¹ h⁻¹) | 5.63 (3.68) | 6.21 (4.55) | 10.04 (2.25) | 10.18 (1.13) |
| | AcPh (μg pNP g⁻¹ h⁻¹) | 29.16 * (2.31) | 38.32 * (4.15) | 61.87 * (16.82) | 89.60 * (21.33) |
| | SPI | 0.76 * (0.07) | 0.63 * (0.03) | 0.36 (0.01) | 0.32 (0.12) |
| | GRSP (μg BSA g⁻¹ dry soil) | 69.8 (4.3) | 69.06 (2.5) | 5.61 (1.9) | 5.17 (1.01) |

S3y: site with three years of CLC application. S1y: site with one year of CLC application. MBC: microbial biomass carbon. MAB: mesophilic aerobic bacteria. MAA: actinobacteria. MFF: filamentous fungi. MPN: most probable number. FDA: fluorescein diacetate hydrolysis. DHA: dehydrogenase. INTF: iodonitrotetrazolium formazan; AlPh: alkaline phosphatase. AcPh: acid phosphatase. pNP: p-nitrophenyl phosphate; SPI: soil pathogenicity index. GRSP: glomalin-related soil protein; BSA: bovine serum albumin. Asterisks indicate significant differences between biofertilized and non-biofertilized plots (** for p<0.05 and * for p<0.1).

Table 4. Matrix of Spearman correlation coefficients (r).

|                       | MBC | MAB | MFF | MAA | FDA | DHA | AlPh | AcPh | Cel | P Sol | NH₄ ox | Den | N fix | SPI | Glom | H |
|-----------------------|-----|-----|-----|-----|-----|-----|------|------|-----|-------|--------|-----|-------|-----|-------|---|
| MAB                   | ns  |     |     |     |     |     |      |      |     |       |        |     |       |     |       |   |
| MFF                   |     | ns  |     |     |     |     |      |      |     |       |        |     |       |     |       |   |
| MAA                   |     | ns  | -0.78** |     |     |     |      |      |     |       |        |     |       |     |       |   |
| FDA                   |     |     |     | ns  | 0.63** |     |      |      |     |       |        |     |       |     |       |   |
| DHA                   |     |     |     | ns  | 0.49* | 0.74** |     |      |     |       |        |     |       |     |       |   |
| AlPh                  |     |     |     | ns  | 0.53** |     |      |      |     |       |        |     |       |     |       |   |
| AcPh                  |     |     |     | ns  | 0.79** |     | -0.64** | 0.81** | 0.72** | 0.58** |     |     |     |       |   |
| Cel                   |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| P-Sol                 |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| NH₄ ox                |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| Den                   |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| N-fix                 |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| SPI                   |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| Glom                  |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| H                     |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |
| pH                    |     |     |     |     |      |     |      |      |     |       |        |     |       |     |       |   |

MBC: microbial carbon biomass. MAB: mesophilic aerobic bacteria. MFF: filamentous fungi. MAA: actinobacteria. FDA: fluorescein diacetate hydrolysis. DHA: dehydrogenase. AlPh: alkaline phosphatase. AcPh: acid phosphatase. Cel: cellulose degraders. P Sol: P-solubilizing microorganisms. NH₄ ox: NH₄⁺ oxidizers. Den: denitrifying microorganisms. N fix: diazotrophs. SPI: soil pathogenicity index. Glom: glomalin. H: humidity. Significant correlations at ** (p<0.05), * (p<0.1), ns (not significant), n=16.
and P, associated with CLC, were important for the definition of PC2. The results also indicated that the differences between the amended and non-amended soils were more pronounced in S3y than in S1y (Fig. 1). For this reason, and for the purpose of establishing the relationships between soil properties after three years of CLC application, another PCA was performed with the data from S3y (Fig. 2). The results indicate (Table 3) that the soil with three years of CLC application was characterized by higher values of N fixation, AcPh enzyme activity, actinobacteria and cellulolytic microorganisms, and lower values of P solubilization, MFF, oxidizing NH$_4^+$, and SPI than NCLC.

Discussion

This research provides insight into how microbial communities function and how composition responds to a short-term (S1y) and long-term (S3y) application of CLC. In accordance with other authors (Giacometti et al., 2014), long-term manure fertilization increases MBC. In this study, MBC was sensitive to CLC application and to the length of time of the application. The results obtained in this study showed that CLC application modifies some soil microbial properties and, in some variables, the effect becomes significant with time. MBC is considered an active component of the soil organic pool. Ren & Stefano (2000) suggested that Cmic:Corg ratios obtained over long-term treatments also represent C equilibrium in the soil. The results obtained at both sites indicate that CLC application increased MBC but not total soil SOC (Table 2). Thus, the changes in soil microbial biomass carbon measured over relatively short periods can indicate the trends in total organic matter content long before these can be detected by chemical analyses. Therefore, MBC as a biological parameter involved in soil C mineralization needs to be monitored together with SOC to predict SOC stability. Although many authors have reported positive correlations among the activities of soil enzymes, the microbial biomass and organic matter contents (Wang et al., 2011), it was not confirmed in this study. NMP increased significantly ($p<0.05$) in both sites with CLC amendments (Table 2). Those differences were not likely due to different soil N content but rather to differences in microbial activity stimulated by CLC application (Qiu et al., 2008) warranting further investigation.

In this study, the abundance of functional groups was influenced by CLC amendments. Organic amendments, including manure application, modify the C/N ratio and the P demand for the development of microbial biomass (Qiu et al., 2008; Luo et al., 2018). CLC application caused increases in NMP of N-fixing organisms and in acid phosphatase enzyme (AcPh) activity. On the other hand, P-solubilizing abundance decreased. Both variables, P-solubilizing and AcPh, are related to microbial activity and P availability, but linked to different soil P pools. P solubilizing was positively correlated with filamentous fungi (MFF) and AlPh to mesophilic aerobic bacteria (MAB) population. This may explain the different activity and origin of P availability in the soil. The majority of the variation in potential enzyme activity and functional groups could be explained by nutrient availability related to CLC application. In both sites, MBC was higher in CLC treatment than in NCLC, but differences were only significant at S1y ($p<0.1$). Soil fertility is related to the activity of functional groups of microorganisms, with the ability to directly or indirectly supply essential plant nutrients because they are linked to N, P and C biogeochemical cycles. Trends in microbial biomass and functionality were not accompanied by a change in the size of the microbial cultivable mesophilic
population of bacteria, fungi and actinomycetes (Table 3). The relationship between inorganic N and soil microbial activity, enzymes or functions is not straightforward because the number of microorganisms represents the number of culturable mesophilic microbial organism and not necessarily the active or functional groups in the soil. In general, microbial activity changes more quickly in response to management than to community composition (Burger & Jackson, 2003). These results indicate that the number of mesophilic aerobic culturable organisms do not show consistent changes with CLC treatment, although quantitative changes of functional groups were recorded in both sites. Cellulolytic microorganisms break cellulose chains into small units and the treatment-enhanced activity of the cellulolytic community could indicate higher litter or root turnover in the soil and therefore a higher C pool. At the same time, the treatment also increases diazotrophic organisms with. An increase in microbial N demand due to CLC fertilization is expected. Furthermore, N₂O and N₂ production is further stimulated by the addition of labile C sources typically added with organic-based fertilizers such as manure (Zhou et al., 2013). Increased N fixation could be a consequence of increased N₂ emission with CLC application (Asgedom et al., 2014; Gao et al., 2016); thus, microbial activity would regulate N input and output from the system. In this study, microbial community composition explained little variation compared to potential enzyme activity and functional groups. The phenotypic plasticity of the microbial community in response to treatment may be high, as suggested by the relatively large variation in soil microbial community functions. There were no statistically significant differences in soil pH among treated and untreated soils (Table 2). Many authors have suggested that manure amendments may decrease soil pH. It is known that soil pH affects microbial biomass and activity, as well as the relative proportions of bacteria and fungi (Pietri & Brookes, 2008, 2009). In this study, soil pH was positively correlated with enzyme activity and bacteria (MAB) and negatively correlated with actinobacteria (MAA) (Table 4). This positive correlation suggests that soil enzyme activity is mainly a function of pH and of the total present MAB population for potential synthesis (Zhou et al., 2019). The soil bacteria community abundance and composition were well adapted to soil pH for optimum enzyme activity (Nannipieri et al., 2017).

The suppressing capacity of soil pathogens (SPI) increased with CLC treatment in both sites. Actinobacteria (MAA) had a strong relationship with SPI, together with glomaline, diazotrophs and denitrifying organisms (p<0.05, Table 4). Actinobacteria have gained special relevance as the most potent source of antibiotics (Kandasamy et al., 2012) and other bioactive secondary metabolites (Solecka et al., 2012) as potential biocontrol agents.

The activities of four enzymes (FDA, DHA, AlPh, AcPh) increased in both sites with the application of CLC. Soil enzyme activities are important indicators of microbiological and biochemical processes because they are involved in soil organic matter decomposition, nutrient cycling and availability, and in the biodegradation of toxic organic pollutants. Recent studies in temperate environments have suggested that measurements of soil enzyme activity are generally the most sensitive indicators of changes in the below-ground microbial community from different management practices (Nanipieri et al., 2002; Chaer et al., 2009; Bowles et al., 2014; Raiesi & Beheshti, 2014). Activities of specific enzymes may change depending on the composition of the organic amendments and the relative availability of nutrients, as well as other factors, such as soil type. Our results indicate that CLC application tends to increase soil enzyme activity and this pattern could be the result of higher microbial activity stimulated by CLC amendment. At the same time, given the relatively constrained level of soil nutrients, enzymatic activity might enhance the availability of the most limiting nutrients in order to meet microbial metabolic demands (Sinsabaugh et al., 2008; Mooshammer et al., 2014), which could
explain the significant increases of AcPh enzyme activity as a result of CLC application in both sites (p<0.05, Table 3). In order to adapt to environmental constraints, microorganisms have to compensate the regulation of extracellular enzyme production with a C and nutrients acquisition strategy or by enhancing microbial metabolism for its nutrient use efficiency. In this study, the AcPh activity (linked to organic P), together with MPN of diazotrophic (N-fixation), celulolitic microorganism (C) and saprophytic fungi (MFF) increased significantly with CLC treatment. This could indicate a microbial response to P organic deficiency and changes of C/N ratio with treatment for the development of microbial biomass. These may partially help resolve the constraint of resource availability and explain the increase in enzyme activity. In this study, all enzyme activities increased; DHA showed a particular increase with CLC treatment in both sites. Among the various soil enzymes, DHA is of great relevance, as its activity levels are considered an indicator of overall microbial activity, due to their intracellular presence in all living microbial cells, and an indicator of biological redox systems.

Contrary to what was expected, our results showed no significant change in GRSP with treatment. According to Zhang et al. (2014), organic amendments enhance soil aggregate stability through the positive effect on soil binding agents, including GRSP. Recent results have described glomalin as a primary soil component in the formation of micro-aggregates and the maintenance of soil structure (Wright & Anderson, 2000; Emran et al., 2012). Results of Wang H et al. (2017) and Wang CY et al. (2017) showed that the enzyme activities after a 23-year manure amendment led to an increase in the macro-aggregates (> 1 mm) but not in the micro-aggregates. The latter could explain the negative correlation between glomalin and enzyme activity obtained in this study. Our results showed that CLC treatment increased enzyme activity and MBC but not total extractable glomalin. This may indicate that microbial biomass and enzyme activity are not linked to glomalin, which is specially related to micro-aggregate formation (Six et al., 2004). Instead, glomalin was positively correlated with MAA and SPI.

In this study, the sampling strategy and microbial parameters used revealed useful information regarding the effects of CLC application at the two sites, and this effect seems to be more evident with time (S1y compared to S3y). This is one of the first studies evaluating the effects of aerobically treated manure application on a large set of soil microbial parameters. The application of CLC had significant effects on biological soil properties, increasing microbial biomass, soil enzyme activity and the abundance of microorganisms of different functional groups. Among the analyzed microbial parameters, microbial biomass carbon (MBC) and soil enzyme activity of tested FDA, DHA, AcPh and SPI emerged as reasonable indicators to assess and monitor the effects of CLC application. Among physicochemical variables, N-mineralization potential (NMP) was the best one to discriminate between treatments. The selected soil microbiological properties and NMP were the most sensitive to CLC amendments, but different soil types or sources of organic amendment applications need further research. With no current consensus on the minimal biological data set to assess the impact of a management practice on soil, this research approach provides insight into how microbial communities function and how composition responds to organic fertilization with CLC and its potential relationship with ecosystem services.

Acknowledgments

The authors would like to thank Andres Peres del Castillo and Christian Decker for their much-appreciated assistance with field work and Sally Bunning for her technical editing of the manuscript.

References

Adam G, Duncan H, 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol Biochem 33: 943-951. https://doi.org/10.1016/S0038-0717(00)00244-3

Altier N, Zerbino S, 2012. Indicadores biológicos para evaluar los agroecosistemas. 4. Comunidades microbianas. Indice de patogenicidad del suelo: Ejemplo para ccuencias con leguminosas forrajeras. In: INIA; Programa Nacional Producción y Sustentabilidad Ambiental. Uso de la biodiversidad para la evaluación del impacto de la intensificación agrícola y el diseño de agroecosistemas sustentables, pp: 73-79. Las Brujas, Canelones (Uruguay).

Asgedom H, Tenuta M, Flaten DN, Gao X, Kebreab E, 2014. Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. Agron J 106 (2): 732-744. https://doi.org/10.2134/agronj2013.0096

Benintende S, Benintende M, Sterren M, Saluzzio M, Barbagelata P, 2015. Biological variables as soil quality indicators: effect of sampling time and ability to classify soils by their suitability. Ecol Indic 52: 147-152. https://doi.org/10.1016/j.ecolind.2014.11.030
Beringer JE, 1974. R Factor transfer in Rhizobium leguminosarum. J Gen Microbiol 84 (1): 188-198. https://doi.org/10.1099/00221287-84-1-188

Bogaard T, Fraser R, Heaton THE, Wallace M, Vaiglova P, Charles M, Jones G, Evershed RP, Styring AK, Andersen NH, Arbogast RM, Bartosiewicz L, Gardeisen A, Kanstrup M, Maier U, Marinaova E, Ninov L, Schafer M, Stephan E, 2013. Crop manuring and intensive land management by Europe's first farmers. P Nat Acad Sci 110 (31): 12589-12594. https://doi.org/10.1073/pnas.1305918110

Bowles TM, Acosta-Martínez V, Calderón F, Jackson LE, 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biol Biochem 68: 252-262. https://doi.org/10.1016/j.soilbio.2013.10.004

Bray RH, Kurtz LT, 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 9 (1): 39-46. https://doi.org/10.1097/00001069-194501000-00006

Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, De Goede R, Fleskens L, Geissen V, Kuypert TW, Mäder P, et al., 2018. Soil quality - A critical review. Soil Biol Biochem 120 (Feb): 105-125. https://doi.org/10.1016/j.soilbio.2018.01.030

Burger M, Jackson LE, 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol Biochem 35 (1): 29-36. https://doi.org/10.1016/S0038-0717(02)00233-X

Casida LE, Klein DA, Santoro T, 1964. Soil dehydrogenase activity. Soil Sci 98 (6): 371-376. https://doi.org/10.1097/00001069-196412000-00004

Chodak M, Nikliška M, 2010. Effect of texture and tree species on microbial properties of mine soils. Appl Soil Ecol 46 (2): 268-275. https://doi.org/10.1016/j.apsoil.2010.08.002

D’Andrea PA, Medeiros MB, 2002 Biofertilizantes biodinâmicos na nutrição e proteção de hortaliças. Congr Bras de Agricultura Organica, Natural, Ecológica e Biodiâmica, 1. Anais. Piracicaba: Agroecológica; Ambrosano E (Coord.).

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW, 2016. InfoStat. Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba. http://www.infostat.com.ar

Dong WY, Zhang XY, Dai X, Fu X, Yang F, Liu X, Sun X, Wen X, Schaeffer SM, 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. Appl Soil Ecol 84: 140-147. https://doi.org/10.1016/j.apsoil.2014.06.007

Dose HL, Fortuna AM, Cihacek LJ, Norland J, Desutter TM, Clay DE, Bell J, 2015. Biological indicators provide short term soil health assessment during sodic soil reclamation. Ecol Indic 58: 244-253. https://doi.org/10.1016/j.ecolind.2015.05.059

Durán A, 1991. Los suelos de Uruguay, 2nd ed. Hemisferio Sur (1985).

Eivazi F, Tabatabai MA, 1977. Phosphatases in soils. Soil Biol Biochem 9 (3): 167-172. https://doi.org/10.1016/0038-0717(77)90070-0

Emran M, Gispert M, Pardini G, 2012. Patterns of soil organic carbon, glomalin and structural stability in abandoned mediterranean terraced lands. Eur J Soil Sci 63 (5): 637-649. https://doi.org/10.1111/j.1365-2389.2012.01493.x

FAO, 1998. World reference base for soil resources. World Soil Resources Report 84.

FAO, 2017. Voluntary guidelines for sustainable soil management. 155th Session of the FAO Council, Rome, Italy, 5th Dec 2016.

Frioni L, 2011. Microbiología básica, ambiental y agrícola, 1st ed. O. Gráfica, Buenos Aires, Argentina.

Gao JQ, 2006. The carbon accumulation and its response to climate change in Zoige Wetland Ecosystem of Qinghai-Tibet Plateau. PhD Dissertation, Inst Geogr Sci Nat Resour Res, Chinese Academy of Sciences.

Gao J, Xie Y, Jin H, Liu Y, Bai X, Ma D, Zhu Y, Wang Ch, Guo T, 2016. Nitrous oxide emission and denitrifier abundance in two agricultural soils amended with crop residues and urea in the North China Plain. PLoS One 11 (5): 1-15. https://doi.org/10.1371/journal.pone.0154773

Gattinger A, Muller A, Haeni M, Skinner C, Fliesbach A, Buchmann N, Mäder P, Stolze M, Smith P, El-Hage Scialabba N, Niggli U, 2012. Enhanced top soil carbon stocks under organic farming. P Natl Acad Sci 109 (44): 18226-18231. https://doi.org/10.1073/pnas.1209429109

Gelderman RH, Beegle D, 1998. Nitrate-Nitrogen. In: Recommended chemical soil testing procedures for the North Central Region; Brown JR (ed.), pp: 17-20. Missouri Agric. Exp. Stn. Pub. No. 221.

Giacometti C, Cavani L, Baldoni G, Ciavatta C, Marzadori C, Kandeler E, 2014. Biological indicators provide information for soil quality assessment of wheat and maize cropping in the highlands of Mexico. Soil Till Res 87: 163-174. https://doi.org/10.1016/j.still.2005.03.005
Influence of manure application on the chemical and microbiological properties of soil

Griffiths BS, Ball BC, Daniell TJ, Hallett PD, Neilson R, Wheatley RE, Osler G, Bohanec M, 2010. Integrating soil quality changes to arable agricultural systems following organic matter addition, or adoption of a ley-arable rotation. Appl Soil Ecol 46: 43-53. https://doi.org/10.1016/j.apsoil.2010.06.012

Huang X, Liu S, Wang H, Hu Z, Li Z, You Y, 2014. Changes of soil microbial biomass carbon and community composition through mixing nitrogen-fixing species with Eucalyptus urophylla in subtropical China. Soil Biol Biochem 73: 42-48. https://doi.org/10.1016/j.soilbio.2014.01.021

Kandasamy S, Muthusamy G, Thangaswamy S, Senthilkumar B, 2012. Screening and identification of antibiotic producing actinomycetes and their antagonistic activity against common pathogens. World Res J Antimicrob Agents 1 (1): 2320-3390.

Leytem AB, Dungan RS, Bjornenber DL, Koehn AC, 2011. Emissions of ammonia, methane, carbon dioxide, and nitrous oxide from dairy cattle housing and manure management systems. J Environ Qual 40 (5): 1383-1394. https://doi.org/10.2134/jeq2009.0515

Luo G, Ling L, Frimon V, Guo J, Guo S, Shen Q, 2018. Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: A meta-analysis. Soil Biol Biochem 124 (May): 105-115. https://doi.org/10.1016/j.soilbio.2018.06.002

McGuire KL, Treseder KK, 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biol Biochem 42 (4): 529-535. https://doi.org/10.1016/j.soilbio.2009.11.016

Mooshammer M, Wanek W, Hämmerle I, Fuchsслeger L, Hofhansl F, Knoltsch A, Schnecker J, Takriti M, Watzka M, Wild B, et al., 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nat Commun 5: 1-7. https://doi.org/10.1038/ncomms4694

Murphy D, Cookson W, Braimbridge M, Marschner, Jones D, Stockdale EA, Abbott LK, 2011. Relationships between soil organic matter and the soil microbial biomass (size, functional diversity, and community structure) in crop and pasture systems in a semi-arid environment. Soil Res 49: 582-594. CSIRO Publishing. https://doi.org/10.1071/SR11203

Muscolo A, Panuccio MR, Mallamaci C, Sidari M, 2014. Biological indicators to assess short-term soil quality changes in forest ecosystems. Ecol Indic 45: 416-423. https://doi.org/10.1016/j.ecolind.2014.04.047

Muscolo A, Settineri G, Attinà E, 2015. Early warning indicators of changes in soil ecosystem functioning. Ecol Indic 48: 542-549. https://doi.org/10.1016/j.ecolind.2014.09.017

Nair A, Ngouajio M, 2012. Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. Appl Soil Ecol 58: 45-55. https://doi.org/10.1016/j.apsoil.2012.03.008

Nannipieri P, Kandeler E, Ruggiero P, 2002. Enzymes activities and microbial and biochemical process in soil. In: Enzymes in the environment: activity, ecology and applications; Burns RG & Dick RP (eds), pp: 1-33. Marcel Dekker, NY. https://doi.org/10.1201/9780203904039.ch1

Nannipieri P, Trasar-cepada C, Dick RP, 2017. Soil enzyme activity : a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. Biol Fertil Soils 54 (5): 11-19. https://doi.org/10.1007/s00374-017-1245-6

Nautiyal CS, 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microb Lett 170 (1): 265-270. https://doi.org/10.1016/S0378-1097(98)00555-2

Nezomba H, Mtambanengwe F, Titttollnelli P, Mapfumo P, 2015. Point of no return? Rehabilitation degraded soils for increased crop productivity on smallholder farms in Eastern Zimbabwe. Geoderma 239: 143-155. https://doi.org/10.1016/j.geoderma.2014.10.006

Nogueira MA, Albino UB, Brandão-Hr O, Braun G, Cruz F, Dias BA, Duarte TD, Gioppo NMR, Menna P, Orlandi JM, et al., 2006. Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in Southern Brazil. Agr Ecosyst Environ 115 (1-4): 237-247. https://doi.org/10.1016/j.agee.2006.01.008

Pagliari PH, Laboski C, 2012. Investigation of the inorganic and organic phosphorus forms in animal manure. J Environ Qual 41 (3): 901-910. https://doi.org/10.2134/jeq2011.0451

Pietri JC, Brookes PC, 2008. Relationships between soil pH and microbial properties in a UK arable soil. Soil Biol Biochem 40 (7): 1856-1861. https://doi.org/10.1016/j.soilbio.2008.03.020

Pietri JC, Brookes PC, 2009. Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. Soil Biol Biochem 41 (7): 1396-1405. https://doi.org/10.1016/j.soilbio.2009.03.017

Preem J, Truu J, Truu M, Mander U, Oopkaup K, Lõhmus K, Helmsaari H, Uri V, Zobel M, 2012. Bacterial community structure and its relationship to soil physico-chemical characteristics in alder stands with different management histories. Ecol Eng 49: 10-17. https://doi.org/10.1016/j.ecoleng.2012.08.034

Qin S, Hu C, He X, Dong W, Cui J, Wang Y, 2010. Soil organic carbon, nutrients and relevant enzyme activities in particle-size fractions under conservational versus traditional agricultural management. Appl Soil Ecol 45: 152-159. https://doi.org/10.1016/j.apsoil.2010.03.007
Qiu S, McComb AJ, Richard W, Bell RW, 2008. Ratios of C, N and P in soil water direct microbial immobilisation - Mineralisation and N availability in nutrient amended sandy soils in southwestern Australia. Agr Ecosyst Environ 127: 93-99. https://doi.org/10.1016/j.agee.2008.03.002

Raiiesi F, Beheshti A, 2014. Soil specific enzyme activity shows more clearly soil responses to paddy rice cultivation than absolute enzyme activity in primary forests of Northwest Iran. Appl Soil Ecol 75: 63-70. https://doi.org/10.1016/j.apsoil.2013.10.012

Ren TZ, Stefano G, 2000. Soil bio-indicators in sustainable agriculture. Sci Agric Sin 33: 68-75.

Rufino MC, Tittonell P, van Wijk MT, Castellanos-Navarrete A, Delve ARJ, de Ridder N, Giller KE, 2007. Manure as a key resource within smallholder farming systems: analysing farm-scale nutrient cycling efficiencies with the NUANCES framework. Livest Sci 112 (3): 273-287. https://doi.org/10.1016/j.livsci.2007.09.011

Saha S, Prakash V, Kundu S, Kumar N, Lal Mina B, 2008. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. Eur J Soil Biol 44 (3): 309-315. https://doi.org/10.1016/j.ejsobi.2008.02.004

Schinner F, Öhlinger R, Kandeler E, Margesin R (eds), 1996. Characteristics of organic soil matter. Methods in soil biology, 1st ed. Springer-Verlag, Berlin Heidelberg. https://doi.org/10.1007/978-3-642-60966-4

Schloter M, Dilly O, Munch JC, 2003. Indicators for evaluating soil quality. Agric Ecosyst Environ 98 (1-3): 255-262. https://doi.org/10.1016/S0167-8809(03)00085-9

Schmidt EL, Belser LW, 1994. Autotrophic nitrifying bacteria. In: Methods of soil analysis. Part 2. Microbiological and biochemical properties, pp: 159-177. SSSA Book Series 5.2, Madison, WI, USA.

Schröder J, 2005. Revisiting the agronomic benefits of manure: a correct assessment and exploitation of its fertilizer value spares the environment. Bioreour Technol 96 (2): 253-261. https://doi.org/10.1016/j.biortech.2004.05.015

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, et al., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11 (11): 1252-1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Six J, Bossuyt H, Degryze S, Denef K, 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till Res 79 (1): 7-31. https://doi.org/10.1016/j.still.2004.03.008

Solecka J, Zajko J, Postek M, Rajnisz A, 2012. Biologically active secondary metabolites from actinomycetes. Open Life Sci 7 (3): 373-390. https://doi.org/10.2478/slll53-012-0036-1

Sparling GP, Feltham CW, Reynolds J, West AW, Singleton P, 1990. Estimation of soil microbial C by a fumigation extraction method - Use on soils of high organic-matter content, and a reassessment of the kec-factor. Soil Biol Biochem 22 (3): 301-307. https://doi.org/10.1016/0038-0717(90)90104-8

Sradnick A, Murugan R, Oltmanns M, Raupp J, Joergensen RG, 2013. Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. Appl Soil Ecol 63: 23-28. https://doi.org/10.1016/j.apsoil.2012.09.011

Sutton M, 2011. Too much of a good thing. Nature Comment 472: 159-161. https://doi.org/10.1038/472159a

Toth SJ, Prince AL, 1994. Estimation of cation-exchange capacity and exchangeable Ca, K and Na contents of soil by flame photometer techniques. Soil Sci 67 (6): 439-446. https://doi.org/10.1007/9001694-19940600-00003

Treonis AM, Erin E, Austin EE, Jeffrey S, Buyer JS, Jude E, Maul JE, Lori Spicer L, Inga A, Zasada IA, 2010. Effects of organic amendment and tillage on soil microorganisms and microfauna. Appl Soil Ecol 46 (1): 103-110. https://doi.org/10.1016/j.apsoil.2010.06.017

Tubiello FN, Salvatore M, Rossi S, Ferrara A, Fitzon N, Smith P, 2013. The FAOSTAT database of greenhouse gas emissions from agriculture. Environ Res Lett 8 (1): 015009. https://doi.org/10.1088/1748-9326/8/1/015009

Vance ED, Brooks PC, Jenkinson DS, 1987. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19 (6): 703-707. https://doi.org/10.1016/S0038-0717(87)90052-6

Walkley A, Black IA, 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37 (1): 29-38. https://doi.org/10.1097/00010694-193401000-00003

Wang Q, Li Y, Wang Y, 2011. Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. Environ Monit Assess 174 (1-4): 241-257. https://doi.org/10.1007/s10661-010-1454-z

Wang CY, Hu N, Ge T, Kuzyakov Y, Wang ZL, Li Z, Tang Z, Chen Y, Wu C, Lou Y, 2017. Fertilization alters microbial community composition and functional patterns by changing the chemical nature of soil organic carbon: A field study in a Halosol. Geoderma 292: 17-24. https://doi.org/10.1016/j.geoderma.2017.01.006

Waring SA, Bremmer JM, 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen
availability. Nature 201 (4922): 951-952. https://doi.org/10.1038/201951a0
Wright SF, Upadhyaya A, 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198 (1): 97-107.
Wright SF, Anderson RL, 2000. Aggregate stability and glomalin in alternative crop rotations for the central great plains. Biol Fertil Soils 31 (3-4): 249-253. https://doi.org/10.1007/s003740050653
Zaccardelli M, De Nicola F, Villecco D, Scotti R, 2013. The development and suppressive activity of soil microbial communities under compost amendment. J Soil Sci Plant Nutr 13 (3): 730-742. https://doi.org/10.4067/S0718-95162013005000058
Zhang X, Wu X, Zhang S, Xing Y, Wang R, Liang W, 2014. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. Catena 123: 188-194. https://doi.org/10.1016/j.catena.2014.08.011
Zhou X, Chen Ch, Wang Y, Xu Z, Duan J, Hao Y, Smaill S, 2013. Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid inner Mongolian grassland. Geoderma 206: 24-31. https://doi.org/10.1016/j.geoderma.2013.04.020
Zhou Z, Yan T, Zhu Q, Bu X, Chen B, Xue J, Wu Y, 2019. Bacterial community structure shifts induced by biochar amendment to karst calcareous soil in southwestern areas of China. J Soil Sedim 19 (1): 356-365. https://doi.org/10.1007/s11368-018-2035-y