

ACTA NOVA**Revista de Ciencias y Tecnología**

^aLaboratorio de Suelos y Aguas,
Universidad Mayor de San Simón, Av.
Petrolera km 5 s/n-

^bLaboratory of Plant and Microbial
Ecology, InBioS, University of Liège,
Botany Bât. B22, Chemin de la Vallée,
4, 4000 Liège, Belgium.

Autor para correspondencia:

Alejandro Coca-Salazar

alej.coca@umss.edu

**Effects of chicken manure management
practices on soil quality and plant
development*****Efectos de las prácticas de manejo del estiércol de pollo en la
calidad del suelo y el desarrollo de las plantas***Alejandro Coca-Salazar^{a, b}, Monique Carnol^b

Resumen: La producción de estiércol de pollo y su uso como fertilizante en la agricultura han aumentado a nivel mundial, sin embargo, los costos asociados a su compostaje con frecuencia conducen a su utilización directa como fertilizante, con potenciales consecuencias para la calidad del suelo y el agua. En las zonas agrícolas de los Andes bolivianos, el estiércol de pollo en distintos estados de compostaje es utilizado como una alternativa para mejorar la fertilidad del suelo. Sin embargo, para formular recomendaciones de manejo adecuadas, es fundamental evaluar sus efectos sobre el suelo y el crecimiento vegetal en diferentes etapas de su descomposición. En este estudio se evaluaron los efectos de diferentes frecuencias de volteo de las pilas de estiércol de pollo (sin volteo y con volteo cada cuatro y dos semanas) sobre su madurez y estabilidad durante 150 días, así como los efectos de su utilización sobre las propiedades del suelo (pH, conductividad eléctrica, fracciones solubles y extraíbles de carbono y nitrógeno, biomasa microbiana y respiración potencial) y el desarrollo vegetal (germinación, longitud de raíces y brotes, producción de biomasa). Se observó una estabilización más rápida del estiércol en las pilas sometidas a eventos de volteo. La fertilización del suelo con estiércol de pollo aumentó el C y nitrógeno extraíbles, la respiración potencial, el pH, la germinación y el desarrollo de *Avena sativa* L. como especie indicadora. El estiércol en etapas tempranas de descomposición provocó daños aparentes en las raíces de plántulas de *A. sativa*, efecto que disminuyó con los eventos de volteo y la maduración del estiércol. Los resultados indican que compostar el estiércol de pollo durante un mínimo de 100 días y con al menos un volteo cada cuatro semanas, asegura una adecuada estabilización y maduración para su aplicación como fertilizante.

Palabras clave: Compostaje de gallinaza, frecuencia de volteo, calidad de suelo, germinación y crecimiento vegetal, agricultura andina.

Abstract: While chicken manure generation and its use as fertilizer have increased worldwide, the cost of composting has frequently led to the direct application with potential negative consequences for soil and water quality. In the agricultural highlands of the Bolivian Andes, chicken manure at varying stages of composting is applied as an alternative for improving soil fertility. However, to formulate management recommendations for its proper use, it is essential to assess its effects on soils and plant growth at different stages of composting. In this study, we evaluated the effects of compost pile turning frequencies (no turning, every four and two weeks) on chicken compost maturity and stability during 150 days, and the effects of the compost produced on soil properties (pH, electrical conductivity, soluble and extractable carbon and nitrogen fractions, microbial biomass and potential respiration), seed germination, root and shoot length, and plant biomass production. Faster manure stabilization was observed in piles subjected to turning events. Soil fertilization with chicken manure increased hot water-extractable C and N, respiration potential, pH, seed germination and growth of a plant species indicator *Avena sativa* L. (shoot length and plant biomass production). The use of manure extracts at early stages of decomposition resulted in apparent root damage to *A. sativa* seedlings, which declined with turning events and compost age. Composting manure piles a minimum of 100 days with one turning event every four weeks before its application to the field ensures proper manure stabilization and maturation.

Key words: Chicken manure composting, turning frequency, soil quality, plant germination and growth, Andean agriculture.

1. Introduction

Over the past decades, world food production has increased steadily in order to meet the growing food demand of the population (Tilman et al., 2002). Much of the increase in food production has been due to higher inputs of chemical fertilizers (Motesharezadeh et al., 2017; Tilman et al., 2002). However, long-term chemical fertilization may cause soil degradation, greenhouse gas emissions, water contamination (Chai et al., 2019; Savci, 2012; Tilman et al., 2001), and affect agricultural production and sustainability in the long run. The alternative to chemical fertilization is the use of organic fertilizers, which improves soil quality for agricultural production by neutralizing soil acidity, increasing carbon and labile nutrient contents, promoting microbial activity and ultimately stimulating healthy root development and plant growth (Duong, 2013). Since the availability of organic fertilizers, such as animal manures, has increased worldwide (Drózdź et al., 2020; Godfray et al., 2010; Kollnig, 2020), their use in agricultural production is considered a solution to reduce the environmental effects of animal wastes (Drózdź et al., 2020) and to face the disruption of chemical fertilizer production and distribution chains, such as those caused by the economic crisis of 2008, the COVID-19 pandemic, or current conflicts in Europe (Berkhout et al., 2022; Hanyabui et al., 2021; Headey and Fan, 2008; Khabarov and Obersteiner, 2017).

Composting is an aerobic process during which organic matter is decomposed and complexed through microbial activity into a nutrient-rich and more stable compounds than the original material. It is divided into four overlapping stages (mesophilic, thermophilic, cooling down and maturation), during which compost properties change, depending on the characteristics of the raw material (e.g. humidity, carbon and nitrogen contents) and the composting practices, such as turning of manure (Bernal et al., 2009). During composting, complexation of labile organic compounds (compost stabilization) and degradation of phytotoxic organic compounds (compost maturation) take place (Khalil and Hassouna, 2013; Raut et al., 2008). Compost management practices such as turning of manure may accelerate these processes by maintaining aerobic conditions, promoting microbial activity (Diaz et al., 1993), while the absence of turning events could slow down the composting process (Khalil and Hassouna, 2013). Despite extensive research conducted on the effects on fully composted manure on soils and plant growth, information on the effects of manure at different stages of decomposition on plant growth and soil quality is limited. However, due to the intense labor required for composting and the increasing demand of organic fertilizers, farmers often apply fresh manure without following recommended management practices (Drózdź

et al., 2020; Kyakuwaire et al., 2019; Ndambi et al., 2019; Yang et al., 2014). The use of fresh manure may lead to potential phytotoxic effects (Bernal et al., 2009; Britto and Kronzucker, 2002; Gajbhiye, 2013), soil contamination with pathogens, antibiotics and trace elements (Deng et al., 2020; Kyakuwaire et al., 2019; Yang et al., 2014), but its high nutrient content (Augstburger, 1983) may improve plant development and increased crop yields. Understanding the effects of manure at different stages of the composting process (from fresh to fully composted) on soils and plant growth are of central importance for contributing to an efficient and optimal use of organic fertilizers.

In the Andean region of Bolivia, potato production has been historically based on three to four cultivation cycles followed by long fallow periods of 10 to 50 years. This was considered a sustainable strategy for managing fertility of soils with low nutrient contents and for achieving high potato yields (Pestalozzi, 2000; Zimmerer, 1993). However, agricultural intensification led to progressive shortening of fallow periods to 2-6 years, and the necessity to incorporate alternative management practices. Soil fertilization has been adopted for managing fertility, but due to the restrictive prize of industrial chemical fertilizers, organic alternatives, such as chicken manure, gained attention due to its low cost, high nutrient content and increasing availability (Augstburger, 1989, 1983; Kollnig, 2020). Currently, chicken manure is the most important fertilizer for potato production in the high mountainous areas and valleys of the country (approximately 165000 ha; INE, 2015). Despite governmental efforts to provided technical support for fertilization, soil fertilization practices rely on communal decisions or farmers' personal judgement (Eguívar and Garcia, 2006) that commonly lead to excessive fertilizer application (FAO, 1999). For example, Augstburger (1989) reported that manure applications should be performed once during a cultivation cycle of three-four years, due to its residual effect. But nowadays farmers report annual fertilizer applications, and additional fertilization events may even take place. Chicken manure management by local farmers is highly variable: from direct use upon delivery to roadside storage in piles during several months. These practices result in fertilization events with manure at different stages of decomposition. As farmers report variable potato yields, root damage and seed germination problems, we hypothesized that these different field observations might be due to the varying composting procedures used for the chicken manure applied to the fields. In particular, we hypothesized that fresh manure might cause phytotoxic effects associated to improper manure stabilization and maturation, impairing seed germination and plant development. We also hypothesized that the application of mature compost would lead to improvement of soil quality for agricultural

production associated with increased labile (soluble, extractable and microbial) C and N, and microbial activity. The aims of this study were to evaluate: (1) the effects of turning frequency on chicken manure compost maturation; (2) the effects of chicken manure compost in different maturity stages produced with or without turning on seed germination and plant growth, and on soil quality for agricultural production.

2. Materials and Methods

2.1 Chicken manure composting

Chicken manure was collected two weeks after the removal of animals from a single broiler house of a typical chicken farm of “Asociación de Avicultores Cochabamba ADA” (poultry farmers association of Cochabamba). The manure originated from broiler chickens raised on floor bedding consisting of wood shavings. Manure composting was performed in the field at Mayor de San Simón University (Bolivia), simulating composting practices of local farmers. Manure was homogenized and used to establish nine piles (length, width and height= 1.5×1×0.7 m, 0.7 m³ each), randomly assigned to three different turning frequency treatments: no turning of the piles (0T), turning every 4 weeks (4W), and turning every 2 weeks (2W). Water holding capacity (WHC) was estimated by the Shaw’s method (Jenkinson and Powlson, 1976), and manure was adjusted to 60% WHC. The composting experiment was considered to start when manure humidity was adjusted. An initial stabilization period of two days was applied to allow moisture redistribution and to ensure uniform initial conditions across piles; therefore, the first sampling at day 2 represents manure with no substantial decomposition but with standardized moisture content. The piles were placed on plastic sheets and covered with plastic film to avoid desiccation. The composting process was followed during 150 days. Manure samples were taken every week during the first three months and then every two weeks until the end of the experiment, resulting in a total of sixteen sampling dates.

At each sampling date, the temperature of the piles was recorded, and six samples (3 kg each) were collected from each pile (two from the top, two from the middle, and two in the lower part of the pile). Samples were homogenized and sieved (4 mm mesh), resulting in one composite sample per compost pile. A sub-sample was stored at 4°C and used for physico-chemical and respiration analyses. The remaining sample was air dried in the dark and stored for the seed germination and plant growth experiments.

Water content of manure samples was determined by weight difference after drying at 105°C for 48 h. pH was determined in a manure-water solution (1:5 w/v) with a

pH-meter (Oakton PC 700). Total organic carbon was determined with the Walkley-Black wet combustion method. Briefly, 6 g manure were mixed with a solution containing 30 ml distilled water, 6 ml sulfuric acid (98%) and 3 ml potassium dichromate, and titrated with ferroin indicator solution (Cáceres, 2012). The total nitrogen content was determined with the Kjeldahl digestion method. Briefly, 0.3 g manure were digested with 6 ml sulfuric acid (98%), distilled with 25 ml sodium hydroxide 10N and NH₃ was collected on a boric acid solution (7%). The ammonia present in the solution was determined through titration with Tashiro methyl red/Methylen blue indicator and sulfuric acid (0.05%) (Cáceres, 2012). The respiration potential was determined as CO₂-C accumulation during 3 h in the headspace of an amber bottle (360 ml) from 5 g fresh manure at 20°C after overnight pre-incubation (Robertson et al., 1999). Gas samples (4 ml) were taken at 0, 120, 150, 180, and 210 min with an airtight syringe (Hamilton Model 1005) and analyzed with an infrared absorption gas analyzer (EGM-4 PPsystem, UK). The respiration potential was estimated by linear regression of CO₂-C against time.

2.2 Seed germination and plant growth experiments

Manure collected from the nine piles was used for seed germination and plant growth experiments. Six of the sixteen sampling dates were selected to cover the whole duration of the composting process and the three turning frequencies 0T, 4W, and 2W. Selected sampling dates corresponded to 2 (mesophilic phase), 23, 45 (thermophilic phase), 46, 107, and 150 (cooling down phase) days of composting. The effect of chicken manure at different stages of decomposition and with varying turning frequencies on seed germination, plant growth and soil quality indicators was thus evaluated through a factorial design with the factors compost age (six levels: 2, 23, 45, 65, 107 and 150 days of decomposition) and turning frequency (three levels: 0T, 4W, and 2W), their combination resulting in a total of 18 treatments (hereafter referred as “manure treatments”).

2.2.1 Effects of chicken manure on seed germination

The effects of manure extracts on seed germination were assessed using certified *Avena sativa* L. seeds as plant species indicator, following ISO 11269-2. Potato was not used because its larger seed size and longer, more variable emergence times do not comply with the validity criteria and timeframes established by the ISO protocol. *A. sativa* seeds were obtained from “Semillas Forrajeras Sociedad Anónima Mixta” (SEFO-SAM) of Mayor de San Simón University. A pre-germination test to evaluate viability of the seed batch indicated its suitability for the experiment (94% germination rate).

Manure extracts were prepared using 5 g dry manure mixed with 50 ml distilled water (1:10 w/v, dry weight basis) and agitated for 1 h at 200 rpm (Gao et al., 2010). Extracts were centrifuged (12000 rpm) and 3 ml were poured in sterile plastic Petri dishes (in triplicate) containing one layer of sterile Whatman #2 filter paper. Ten seeds were placed into each Petri dish and covered with a layer of thin Whatman #2 filter paper to ensure that the whole seed surface was in contact with the solution. For each of the 18 manure treatments (3 turning treatments, 6 sampling dates), three replicate Petri dishes were established, resulting in a total of 54 Petri dishes that were incubated at 23°C for three days. Three control Petri dishes containing seeds and distilled water were included. The number of germinated seeds, and the number of germinated seeds showing apparent root damage in the form of tissue orange (Supplementary material S1) were recorded.

2.2.2 Effects of chicken manure on plant growth

We tested the effect of soil fertilization with manure at different stages of decomposition and with varying turning frequencies on germination and growth of *A. sativa* as plant species indicator, and on soil quality indicators following ISO 11269-2 (2012).

Soil was collected from a 4-year fallow field located in Chullchunqani community, a traditional agricultural community that produces potato as their main crop located in the central Andean region of Bolivia (Pocona municipality, Cochabamba). Fallow soil was chosen for the experiment, as the use of soil from crop fields may lead to potential residual effect of the last fertilization event, which can last to up to two years (Condori et al., 1997). The 0.4 ha field was divided into 10 m² quadrants and ten quadrants were randomly selected. One soil sample was collected (20 cm depth) in the middle of each quadrant, and all samples were bulked, homogenized, air dried, and sieved (4 mm).

For each of the 18 manure treatments (3 turning treatments, 6 sampling dates), 2.4 g dry manure of each pile was mixed with 450 g dry soil (0.6%) in triplicate (54 samples in total). The manure:soil ratio was determined through a poll conducted with farmers of the Pocona municipality (n=21) to determine the average quantity of manure applied per hectare at a depth of 0.2 m. Surveyed farmers responded specifically in the context of potato fertilization, thus, the proportion applied in this study reflects local potato fertilization practices. Soil-manure mixtures were adjusted to 80% water holding capacity and placed into plastic pots. Three control pots containing soil without manure, and three technical controls containing ultra-pure sand were also included in the experiment. In each pot, ten *A. sativa* seeds were planted (10-15 mm depth) spaced uniformly.

Pots were randomly placed in a greenhouse and rearranged twice a week during the experiment. Soil humidity was maintained daily by recording pots' weight and adding the required amount of deionized water. The pots containing sand were watered with a nutrient solution (Supplementary material S2) to avoid nutrient deficiencies. The start of the experiment was determined by the emergence of 50% of seedlings in the control pots (containing not fertilized soil). At this moment, the number of emerging seedlings was recorded, and five seedlings were kept in each pot to reduce potential competition between plants. Seedlings were allowed to grow for 15 days and were then carefully removed from the pots (the experiment lasted a total of 24 days). Root and shoot length of each plant were recorded, all plants were dried (70°C for 48 h) and weighed.

2.2.3 Effects of chicken manure on soil characteristics

The remaining soil from the plant growth experiment was stored at 4 °C and analyzed for soil physico-chemical characteristics. Soil pH was determined with a pH-meter meter (HI2550 HANNA instruments) in 15 g fresh soil in a KCl 1M solution (1:2 w/v; Allen, 1989). Water soluble and hot water-extractable carbon and nitrogen were determined using a sequential extraction. Water soluble carbon and nitrogen were extracted from 7.5 g fresh soil with 45 ml distilled water at room temperature (Ghani et al., 2003). The soil solutions were agitated (120 rpm, 30 min) centrifuged (4000 rpm, 10 min), and the supernatants were filtered and stored for chemical analyses. The remaining soil was re-suspended in 45 ml distilled water, and placed in an oven at 80 °C for 16 hours to determine the hot water-extractable carbon and nitrogen (Ghani et al., 2003). Solutions were agitated and centrifuged as described above. Water soluble carbon (WSC) and nitrogen (WSN), and hot water-extractable carbon (HWC) and nitrogen (HWN) of the solutions were determined with a total organic carbon analyzer (Analytik Jena Multi N/C 3100 TOC/TN Analyzer).

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined through fumigation-extraction method (Anderson and Domsch, 1978). Fumigation of soil subsamples was carried out for 3 days in a vacuum desiccator with alcohol-free chloroform. Ten grams of fumigated and non-fumigated samples were extracted with 50 ml 0.5 M K₂SO₄ (1 h shaking at 180 rpm and filtration through Whatman filter #42). Organic carbon and total nitrogen in the extracts were measured with a total organic carbon analyzer (Analytik Jena Multi N/C 3100 TOC/TN Analyzer). The respiration potential was determined as CO₂-C accumulation during 3h in the headspace of an amber bottle (125 ml) from 20 g fresh soil, at 20 °C in the dark after an overnight pre-incubation (Robertson et al., 1999), as described above.

2.3 Statistical analysis

We evaluated initial manure characteristics (2 days of decomposition) of the three turning frequencies 0T, 4W and 2W with one-way ANOVA and Tukey tests on the variables measured. The effect of turning frequency (0T, 4W and 2W) on manure composting was evaluated by fitting linear models against composting time for each replicate for pH, TOC, TN, and C:N, and non-linear asymptotic convex models for respiration potential for each manure pile during the composting period. General additive models were used for temperature with smoothing function and 4 knots for the explanatory variable days (Zuur et al., 2009). One-way ANOVA and Tukey post-hoc tests were conducted on the slopes for the linear models, on the minimum attainable value for respiration potential, and on the maximum average value for temperature.

Changes in seed germination, plant growth and soil characteristics compared to control soil (not fertilized) were assessed through Dunnett's test. Two-way ANOVAs were used to assess the effects of compost age, turning frequency and their interaction. Tukey post-hoc tests were used for assessing the differences between manure treatments.

All analyses were conducted in the R software (R Core Team, 2018), using the packages "car" (Fox et al., 2018), "dplyr" (Wickham et al., 2019), "lsmmeans" (Lenth, 2016), "mgcv" (Wood, 2017), "multcomp" (Hothorn et al., 2017), "propagate" (Spiess, 2018), "userfriendlyscience" (Peters, 2018).

3. Results

3.1 Changes in manure characteristics during composting

There were no significant differences in the initial temperature, pH, TOC, TN, C:N ratio and respiration potential between the compost piles assigned to the three turning frequency treatments (Table 1). Temperature, TN, C:N ratio and respiration potential changed over the course of the composting process (Figure 1). The average temperature of the manure piles at the beginning of the experiment was 39-40°C. Maximum average temperatures were reached after one month of decomposition, ranging between 45.0 and 46.8 °C, followed by a decrease in temperature until the end of the composting process, when temperatures reached an average of 31.0 °C for the three turning frequency treatments. Total nitrogen content in the manure of piles turned every 4 and 2 weeks decreased at constant rates of -0.07 and -0.08 g kg⁻¹ day⁻¹, respectively, whereas the 0T treatment showed a significantly higher rate (-0.02 g kg⁻¹ day⁻¹, slope not significantly different from 0, *p*-value > 0.05). The C:N ratio of the manure piles of the 4W and 2W treatments increased linearly at a rate of 0.04 and 0.06 units day⁻¹, respectively, while 0T had significantly lower rate change of 0.01 units day⁻¹ (slope not significantly different from 0, *p*-value > 0.05). The initial respiration potentials of 0T, 4W and 2W were 216.9±38.8, 223.5±49.2 and 227.9±43.6 µg C-CO₂ h⁻¹ g⁻¹, respectively, and decreased during the first fifty days of decomposition until they reached the minimum attainable respiration potentials according the fitted models of 30.4±3.7 for 0T, 21.3±2.7 for 2W and 14.3±2.2 µg C-CO₂ h⁻¹ g⁻¹ for 4W, with significantly higher values for 0T compared to 2W and 4W.

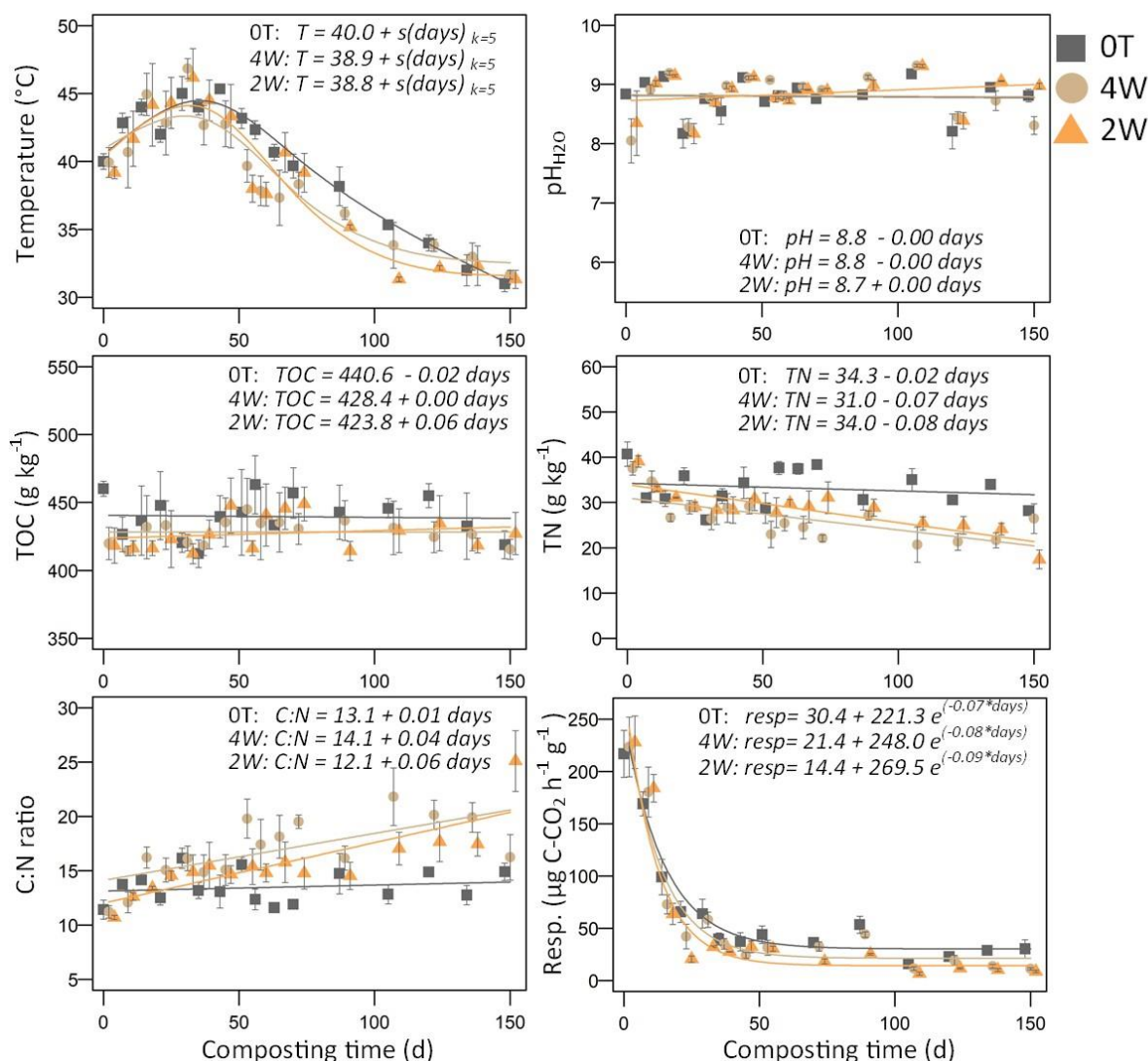
Table 1. Manure characteristics at the beginning of the composting period (2 days of composting) for three turning frequencies: 0T = no turning of the piles, 4W = turning every 4 weeks, 2W = turning every 2 weeks. Mean and standard deviation are presented (n=3). There were no significant differences between the piles assigned to the different treatments (ANOVA and Tukey tests, *p*-value<0.05). pH_{H2O}: pH measured in water; TOC: total organic carbon; TN: total nitrogen; C:N ratio: carbon-to-nitrogen ratio.

Parameter	0T	4W	2W
Temperature (°C)	40.0±1.0	39.9±1.9	39.2±0.76
Water content (%)	61.6±8.6	64.2±1.7	65.8±3.0
pH _{H2O}	8.8±0.1	8.0±0.6	8.3±0.9
TOC (g kg ⁻¹)	460.2±9.5	419.9±20.5	418.3±22.5
TN (g kg ⁻¹)	40.7±4.6	37.5±2.6	39.1±2.2
C:N ratio	11.4±1.5	11.2±1.0	10.7±0.3
Respiration potential (µg C-CO ₂ h ⁻¹ g ⁻¹)	216.9±38.9	223.5±49.2	228.0±43.6

There were no significant changes in pH and TOC of the manure piles during the composting period (slopes of the

three treatments did not differ from 0, p -value > 0.05, Figure 1).

Figure 1. Evolution of the manure characteristics (temperature, pH, TOC: total organic carbon, TN: total nitrogen, C:N ratio, and Respiration: respiration potential) over 150 days of composting. For each sampling time we present the mean and standard deviation of the turning frequency treatments: OT = no turning of the piles; 4W = turning every 4 weeks; 2W = turning every 2 weeks. Each fitted line represents the averaged model ($n=3$) for each of the turning frequency treatments. General additive models were fit for the evolution of temperature where a smoothing function with 5 knots was applied for “days”. Linear models were fit for pH, TOC, TN and C:N ratio, and negative asymptotic model were fit for respiration potential.



3.2 Effects of chicken manure on seed germination, root damage and plant growth

Water extracts of chicken manure of different composting ages and prepared with different turning frequencies did not have any effect on germination of *A. sativa* seeds (Figure 2). The percentage of seeds germinated in manure extracts did not differ from the percentage of seeds germinated in water (p -value > 0.05). However, seeds germinated in manure extracts had significantly higher

percentage of apparent root damage in comparison to seeds germinated in water for the fifteen treatments (combinations of turning frequency and compost time). Only apparent root damage of seeds germinated in extracts of manure sampled at 65 and 107 days of composting turned every 4 weeks did not significantly differ from seeds germinated in water, as well as extracts of manure sampled at 150 days of composting turned every 2 and 4 weeks (Figure 2).

Compost age had a significant effect on the percentage of germinated seeds that had apparent root damage (Figure 2). Seeds germinated with extracts of manure sampled at 2 and 23 days of composting had the highest percentage of root damage, ranging between 47 and 53%. Seeds germinated on extracts of manure sampled at 150 days of composting had the lowest percentage of root damage, ranging from 9 to 24%. Although the two-way ANOVA test indicated a significant effect of turning frequency on apparent root damage, the post hoc Tukey test did not detect significant differences between turning frequency treatments.

Fertilization of soil with chicken manure composted for 2 to 150 days and prepared with different turning frequencies did not have any significant effect on seed germination, shoot length, and biomass production of *A. sativa* (two-way ANOVA, p -value>0.05, Figure 3). Although

two-way ANOVA indicated that compost age had a significant effect on root length (p -value = 0.04), Tukey post hoc comparison did not detect differences between manures sampled at different stages of composting (Figure 3). In comparison to the control (non-fertilized soil), the addition of manure to the soil had a significant effect on seed germination, shoot length and plant biomass (dry matter). Seven treatments that received manure showed higher number of germinated seeds in comparison to control soils. *A. sativa* plants grown in soils that received manure sampled along the composting process (from 2 to 150 days of composting) had longer shoots and produced significantly more biomass in comparison to plants grown in non-fertilized soils. The addition of manure did not affect the root length of plants grown in fertilized soil in relation to those grown in control soil.

Figure 2. Effects of extracts of chicken manure at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeks, 2W: turning every 2 weeks) on seed germination and apparent root damage. For each treatment the mean and standard deviation are presented (n=3). Asterisks indicate significant difference in comparison to control seeds germinated in water (Dunnett's test, p -value<0.05). Different letters indicate significant differences between compost age (two-way ANOVA and Tukey tests, p -value<0.05).

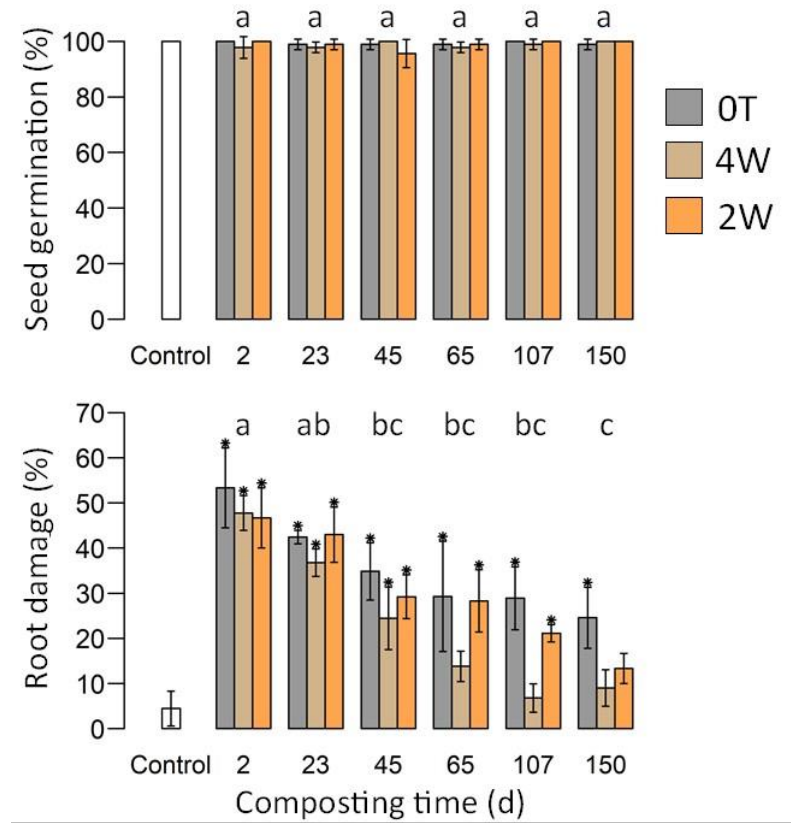
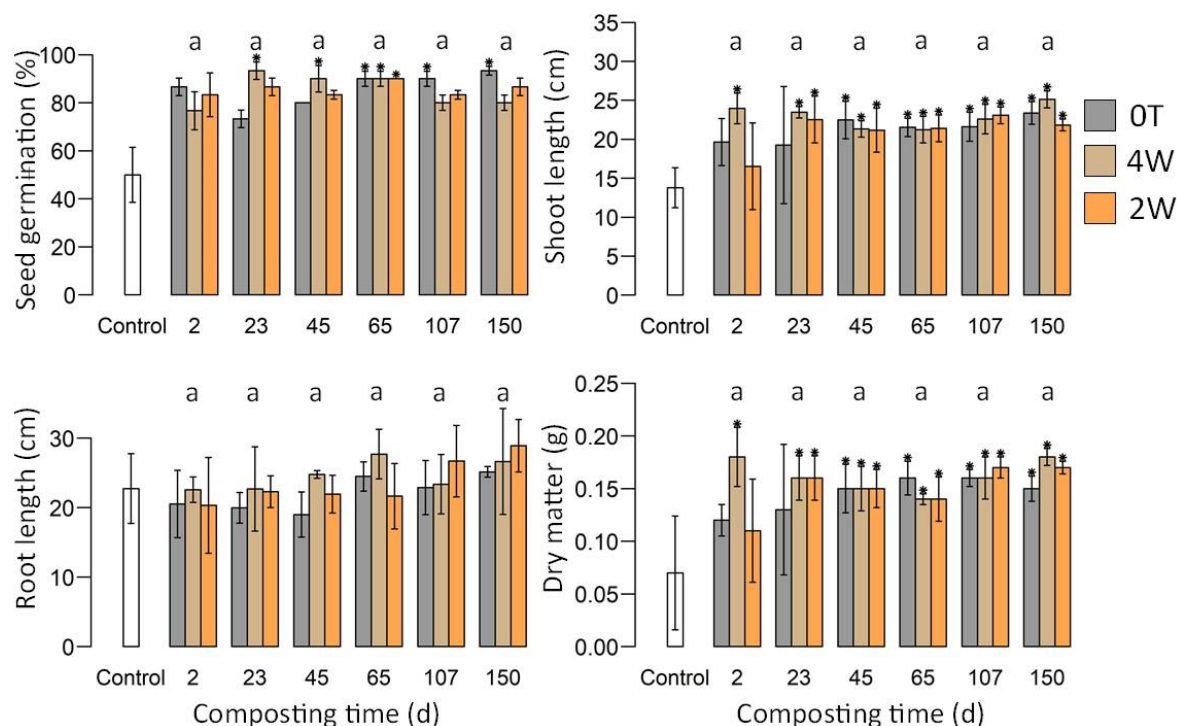


Figure 3. Effect of chicken manure fertilization of soils on seed germination, shoot and root lengths and dry matter of *A. sativa* root and shoots. Manures were at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and prepared with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeks, 2W: turning every 2

weeks). For each treatment the mean and standard deviation are presented ($n=3$). Asterisks indicate significant difference in comparison to non-fertilized control soil (Dunnett's test, p -value <0.05). Different letters indicate a significant differences between compost age (two-way ANOVA and Tukey tests, p -value <0.05).



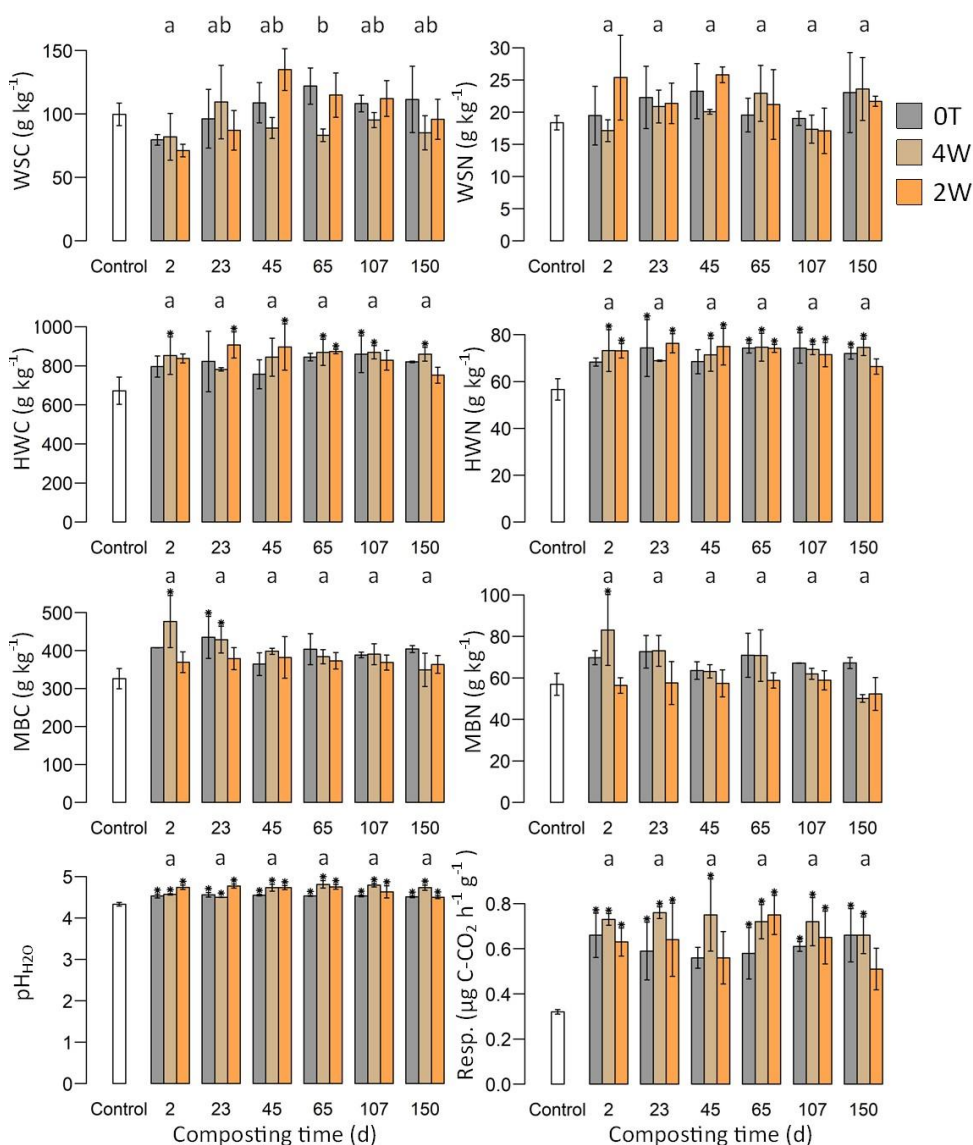
3.3 Effects of fertilization on water soluble and extractable C and N, and respiration potential

The addition of chicken manure of different compost ages and prepared with different turning frequencies showed a significant interaction effect on WSC content and soil pH_{KCl} (two-way ANOVA, p -value <0.05 , Figure 4). Comparison between soil treatments fertilized with manure of different composting time indicated that the lowest WSC values were recorded at 2 days of composting and the highest values were recorded after 65 days of composting, whereas no differences were observed for soil pH_{KCl} . Compost age had a significant effect on MBC and MBN (two-way ANOVA, p -value <0.05), however Tukey tests did

not detect differences between soils fertilized with manure of different compost age (Figure 4). Turning frequency was significant for MBC and MBN, with the manure piles turned every 2 weeks showing the highest MBC and MBN values (supplementary material S3). For respiration potential, turning frequency was also significant but the Tukey test did not detect differences between turning frequency treatments (supplementary material S3). Composting time did not have a significant effect on respiration potential. Fertilization of soil with chicken manure of different compost age and prepared with different turning frequencies did not have an effect on WSN, HWC and HWN (two-way ANOVA, p -value >0.05 , Figure 4).

Figure 4. Effect of fertilization with chicken manure on water soluble carbon (WSC), water soluble N (WSN), hot water-extractable C (HWC), hot water-extractable N (HWN), microbial biomass C (MBC), microbial biomass N (MBN), pH and respiration potential (Resp.). Manures were at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and prepared with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeks, 2W: turning every 2 weeks). For each treatment the mean and standard deviation are presented ($n=3$). Asterisks indicate significant difference in

comparison to non fertilized soil (Dunnett's test, p -value<0.05). Different letters indicate a significant differences between compost age (two-way ANOVA and Tukey tests, p -value<0.05)



In comparison to control soil, the addition of chicken manure led to a significant increase in HWC of eight treatments (Figure 4). HWN also increased in comparison to control soil, except for the soils receiving treatment 0T at two and 45 days of decomposition, and the soil receiving manure of treatment 2W at 150 days of decomposition. The addition of manure sampled at 2 and 23 days of composting resulted in significantly higher MBC and MBN in comparison to control soils. Similarly, the addition of chicken manure had a significant effect on respiration potential. For most of the incubation treatments respiration potential increased in comparison to control non-fertilized soils, except for 0T and 2W turning frequency treatments at 45 days of decomposition, and the 2W turning frequency treatment at 150 days of decomposition.

4. Discussion

4.1 Effects of turning frequency on compost stability

We observed the four phases of composting based on changes in compost temperature: mesophilic, thermophilic, cooling down, and maturation phase. The highest average temperatures recorded ranged between 45 and 47 °C, which may not be high enough for proper manure sanitation, since a temperature between 50 and 55 °C is required for elimination of pathogens (Rynk, 1992). Nevertheless, improper manure sanitation may not necessarily lead to potato diseases, as the abundance of soil-borne pathogens reported in this agricultural region

(i.e. nematodes *Nacobbus aberrans*, and *Globodera* spp.; Franco et al., 1999) may be restricted by chicken manure applications (Cristobal-Alejo et al., 2006; Rodríguez-Kábana, 1986). However, the presence of other potato pathogens of bacterial and fungal origin should not be ruled out, as their survival and transmission depend on specific environmental and management conditions. The temperature of piles turned every 4 or 2 weeks decreased more rapidly than without turning, and stabilized already after 100 days of composting, indicating faster manure stabilization. This is in accordance with previous studies showing that turning of compost piles accelerates decomposition by improving aeration, and consequently microbial activity (Khalil and Hassouna, 2013). After 150 days of composting, turned and non-turned compost piles did not show any difference in the temperature. Its stabilization in the turning frequency treatments indicated that the end of the bio-oxidative decomposition had been reached, and that the compost was stable (Iglesias Jiménez and Perez Garcia, 1989).

Despite the fact that temperature is a convenient measurement for assessing the progression of the composting process, it lacks of precision for assessing manure stabilization, due to variability in heat loss (Willson and Dalmat, 1986). In contrast, microbial respiration provides a direct indication of the quantity of easily degradable organic compounds in the manure, and high respiration rates are associated with the lack of compost stability (Gao et al., 2010; Willson and Dalmat, 1986). In our study, the respiration potential of the manure piles decreased rapidly during the first month of decomposition, indicating the degradation of most labile organic matter. Manure piles then reached the minimum attainable respiration potential, suggesting manure stabilization (Gao et al., 2010). The lower minimum attainable respiration potential rates reached in the turned piles indicated that turning events led to a more efficient composting process. Mechanical disruption of the piles during turning events likely increased accessibility of labile carbon to microorganisms and promoted aerobic conditions that favored microbial activity and organic matter decomposition. Similar results have been previously reported, and compost aeration was suggested to play a central role in manure stabilization and reduction of compost phytotoxic effects (Carballo et al., 2009; Nada, 2015). Such phytotoxic effects are commonly associated not only with poor aeration, but also with high concentrations of ammonium/ammonia, accumulation of organic acids and phenolic compounds, excessive salinity, and pH imbalances in the compost, all of which tend to decline as compost maturation progresses. Concomitantly, we observed a significant reduction of root damage of seeds germinated in turned piles compared to non-turned piles (see below).

Despite the intense labor required for composting, the practice of turning the chicken manure piles should be promoted among farmers to accelerate stabilization and maturation. In fact, the organizational structure of the agricultural communities in the Andean region of Bolivia offers an opportunity to implement proper compost management practices as these communities are organized around collective decision-making of potato production and natural resource management (Ellis-Jones and Mason, 1999; Vargas, 2017). Concomitantly, the current family-based manure management could transition toward larger-scale communal management, enabling the implementation of proper composting practices and labor optimization to ensure the provision of stabilized and mature manure.

We observed a decrease in total nitrogen concentrations of turned compost, without a change in total carbon concentrations, during the 150 days of composting, leading to an increase of the C:N ratio. Commonly the C:N ratio of composted material tends to decrease over time (Khalil and Hassouna, 2013) but we observed the opposite trend in the turned piles, which could be due to increased evaporation of NH_3 in this alkaline environment (de Guardia et al., 2008). In addition to alkalinity, excessive moisture coupled with intense microbial activity could have led to anoxic microsites, favoring gaseous N losses through denitrification (Hao and Chang, 2001). Accordingly, estimates indicate that up to 20% of the total of N loss from chicken manure is released to the environment in the form of ammonia (Drózdź et al., 2020), which could have been exacerbated by turning events. Likewise, a relatively low initial C:N ratio of the manure may predispose the system to an apparent increase during composting, since nitrogen losses through volatilization or denitrification can occur more rapidly than carbon losses, thereby increasing the ratio despite ongoing decomposition.

4.2 Effect of chicken manure on seed germination and plant growth

Effects of chicken manure were tested with compost sampled during the mesophilic, thermophilic, cooling down, and maturation phases. The application of chicken manure at different stages of composting to soils, at a rate typically applied by the local farmers, did not lead to differences in seed germination, root and shoot length. Extracts of chicken manure at different stages of composting also did not have any effect on seed germination but caused apparent root damage in the form of tissue orangeing. Tissue orangeing is generally attributed to cation-anion imbalances and soil acidification in the rhizosphere zone, and it has been widely reported in plants exposed to high ammonium/ammonia concentrations (above 0.1 to 0.5 mmol of NH_4^+ per L; Britto

and Kronzucker, 2002; Westwood and Foy, 1999). High amount of salts in manure extracts could have also contributed to the observed effects on roots, associated to osmotic and ionic stress caused by high concentrations of Na^+ , Mg^{2+} , Cl^- , and SO_4^{2-} (Baligar et al., 1998; Li-Xian et al., 2007). Apparent root damage showed a decreasing trend with increasing compost age, indicating compost maturation, likely due to the decrease of $\text{NH}_4^+/\text{NH}_3^-$ or to the degradation of potential phytotoxic compounds, such as organic acids and phenols (Riffaldi et al., 1986). Moreover, turning of the piles reduced apparent root damage to similar levels than those recorded in control non-fertilized soils after 64 days of composting, indicating faster compost maturation associated to this practice. This beneficial effect on compost quality coexisted with the observed nitrogen losses and increase of the C:N ratio in turned piles, highlighting that turning accelerates stabilization and reduces phytotoxicity, although at the expense of higher N volatilization. These results are in accordance with previous studies indicating faster decrease of phytotoxicity for aerated composts (Carballo et al., 2009; Riffaldi et al., 1986). Consequently, while manure extracts at early stages of decomposition caused apparent root damage, soil fertilization with chicken manure had a positive effect on plant development (seed germination, shoot length, biomass production). Improved development of plants grown in fertilized soils indicated that manure addition contributed to soil quality, likely by increasing organic matter and micronutrient contents, reducing soil acidity, and improving water availability to plants (Mann et al., 2002; Mondo et al., 2012).

Despite the apparent root damage on seeds germinated in manure extracts, we did not observe a decrease in root length when the compost at different stages of maturation was applied to the soils. This could be due to a dilution effect upon soil fertilization and to the adsorption of phytotoxic compounds to soil organic matter or to their degradation by soil microorganisms (Xiao et al., 2020). While these findings reflect responses of an ISO-recommended indicator species and soil-process dynamics, direct extrapolation to effects on *S. tuberosum* should be made with caution due to its distinct physiology compared to *A. sativa*. Nevertheless, under field conditions plant responses can differ substantially, as manure applications are not homogeneous and furrow fertilization (chicken manure applied to the furrow in direct contact with potatoes at sowing time) is a common practice that may lead to locally high manure concentrations. Although furrow fertilization is considered efficient method to increase nutrient availability to plants (Beukema and van der Zaag, 1990), the high manure concentration around potato seedlings may increase the risk of phytotoxic effects from immature manure or high ammonium/ammonia concentrations (Hoover et al.,

2019). This may explain the occasional occurrence of “root burning” reported by local farmers, based on testimonies gathered during fieldwork interviews. Furthermore, additional fertilization events may take place upon plant emergence or flowering, leading to potential over fertilization (FAO, 1999). The farmers’ rationale is that increased manure application would lead to higher potato yields, but increased manure additions are not coupled with linear increases in crop yield (Liu et al., 2021). Despite the fact that fertilization is essential for potato production, over-fertilization during the vegetative developmental stage may lead to excessive haulm growth instead, which delays tubers formation, bulking and maturation (Alva, 2004; Beukema and van der Zaag, 1990). The risk of losses during storage and transportation increases if immature potatoes are harvested because they are easily damaged (Beukema and van der Zaag, 1990). Also, excess fertilization may promote secondary tubers formation, affecting nutrient allocation and leading to the production of several small tubers (Beukema and van der Zaag, 1990) less valuable in the market.

Homogenized manure application is an alternative to reduce the risk of locally high manure concentrations and its associated effects (i.e. manure phytotoxicity, NH_4^+ toxicity, contamination of ground water with NO_3^-), however, the cost of equipment required for manure homogenous fertilization may be restrictive for most farmers. Split fertilization may be a suitable and cheaper alternative to reduce the risk of locally high manure concentrations that can be combined with the traditional furrow fertilization. Moreover, soil nutrient testing is essential to determine optimal fertilization regimes based on plant needs, and to reduce the risk of over-fertilization, to improve nutrient use efficiency, and to reduce economic production cost.

4.3 Effect of chicken manure on indicators of soil quality

Fertilization of soil with chicken manure increased the amounts of hot water-extractable C and N. These C and N fractions are considered to provide readily available nutrients for microorganisms and plants (Chodak et al., 2003; Curtin et al., 2021), and they likely contributed to the higher microbial activity observed. These results are in accordance with previous studies indicating that fertilization increases labile carbon fractions (Darby et al., 2006). Due to the close link between labile pools and soil fertility, increases in the labile hot water-extractable C and N are indicative of increased nutrient availability to plants (Anderson and Domsch, 1980; Ghani et al., 2003), and therefore improved soil quality for cultivation. Moreover, soils with high levels of labile organic matter have complex soil microbial communities that could promote plant

growth and suppress the activity of soil-borne pathogens (Aryantha et al., 2000).

Microbial biomass increased in soils fertilized with manure at mesophilic and thermophilic phases of decomposition (2 and 23 days of composting), which is likely explained by the labile organic compounds present in not stabilized manure that promoted microbial growth. Surprisingly, the use of manure at mesophilic and thermophilic composting stages did not lead to higher soil respiration potential rates. This could be attributed to changes in the energy maintenance demands and C allocation in structural compounds rather than respiration, however, we did not observe differences in the quantity of respired CO₂-C per unit of microbial biomass (an indicator of energy maintenance demands; Anderson and Domsch, 1989, data not shown).

As the decomposition process advances, labile organic compounds present in chicken manure are degraded by microorganisms, and the effect on microbial biomass decreases. On the other hand, respiration potential increased in response to manure fertilization independent of compost age, suggesting that this measurement is more sensitive to C additions than microbial biomass. Also, the short-term response of soil microorganisms to labile organic inputs (Poll et al., 2007) could have influenced the detection of differences between treatments. It is thus possible that microbial biomass could have increased right after fertilization and gradually declined during the plant growth experiment. Additional factors may have also contributed to the observed response of soil microorganisms to manure additions, such the microbial community composition or ecological interactions with plants and nematodes (Faissal et al., 2017; Liang et al., 2020; Poll et al., 2007).

The decrease of soil acidity could be due to the high pH of the manure added to the soil, but previous studies also indicate that manure applications increase soil organic matter, which acts as pH buffer and increases nutrient content (Mann et al., 2002; Mondo et al., 2012; Soremi et al., 2017). Less acidic conditions may favor plant growth, particularly potato production, since drastic potato yield reductions have been reported pH values below 4.6 (Lierop et al., 1982). Altogether these results suggest improvement of soil quality for agricultural production associated to chicken manure fertilization, but different management practices of manure composting (turning events and compost age) did not lead to significant differences in the soluble and extractable C and N fractions, microbial activity and soil pH.

5. Conclusions

Chicken manure piles subjected to turning events showed an acceleration of the decomposition process, more

efficient stabilization and faster maturation. Chicken manure fertilization had a positive effect on soil quality for agricultural production and plant development. Fertilization increased hot water-extractable C and N, respiration potential, and soil pH compared to non-fertilized soil, but the use of manure with different turning frequencies and with different composting time did not lead to significant differences on soil properties. Concomitantly, seed germination, shoot length, and biomass of the indicator species *A. sativa* increased compared to non-fertilized soils. Nevertheless, we observed phytotoxic effects of immature manure extracts on *A. sativa* roots, which showed a decreasing trend with increasing compost age and more turning events. Application of manure at earlier stages of decomposition (not stabilized and immature manure) should be avoided, a minimum of 100 days of decomposition and turning manure piles every four weeks is suggested instead, to ensure proper manure stabilization, maturation and to reduce the risk of phytotoxic effects on plants.

Acknowledgements

We thank the farmers of Chullchunq'ani community, particularly to Albina Maldonado, for allowing sampling her fallow fields. We thank José Luis Capa and Hugo Frolli for conducting the composting and greenhouse experiments, respectively. The authors also thank Teresa De la Cruz for transporting the soil samples to Belgium and to Alfredo Cáceres, Gino Aguirre, Emigdio Céspedes, Arnaud Degueldre, Assia Tahiri and Bernard Bosman for their scientific/technical support.

Funding: This work was funded by the Académie de Recherche et d'Enseignement Supérieur (ARES, Belgium) and Dirección de Investigación Científica y Tecnológica of Universidad Mayor de San Simón (DICYT-UMSS, Bolivia).

Author contributions

Alejandro Coca-Salazar: conceptualization, field work, data analysis, writing (original draft, review and editing). Monique Carnol: conceptualization, data analysis (supporting), funding acquisition, writing (review and editing).

References

- Allen, S.E., 1989. Chemical analysis of ecological materials, 2nd ed. Blackwell Scientific Publications, Oxford, UK.
- Alva, L., 2004. Potato Nitrogen Management. *Journal of Vegetable Crop Production* 10, 97–132. doi:10.1300/J068v10n01_10
- Anderson, J.P., Domsch, K.H., 1980. Quantities of plant nutrients in the microbial biomass of selected soils. *Soil*

- Science 130, 211–216. doi:10.1097/00010694-198010000-00008
- Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* 10, 215–221. doi:10.1016/0038-0717(78)90099-8
- Anderson, T.H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biology and Biochemistry* 21, 471–479. doi:10.1016/0038-0717(89)90117-X
- Aryantha, I.P., Cross, R., Guest, D.I., 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. *Phytopathology* 90, 775–782. doi:10.1094/PHYTO.2000.90.7.775
- Augstburger, F., 1989. Abonos organicos en el cultivo de la papa en la zona andina de bolivia. Ficha técnica 19 14.
- Augstburger, F., 1983. Agronomic and economic potential of manure in Bolivian valleys and highlands. *Agriculture, Ecosystems and Environment* 10, 335–345. doi:10.1016/0167-8809(83)90084-1
- Baligar, V.C., Fageria, N.K., Elrashidi, M.A., 1998. Toxicity and nutrient constraints on root growth. *HortScience* 33, 960–965. doi:10.21273/hortsci.33.6.960
- Berkhout, P., Bergevoet, R., Berkum, S. Van, 2022. A brief analysis of the impact of the war in Ukraine on food security, Policy Document 2022-033. Wageningen Economic Research, Wageningen. doi:10.4060/cb9171en
- Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology* 100, 5444–5453. doi:10.1016/j.biortech.2008.11.027
- Beukema, H.P., van der Zaag, D.E., 1990. Introduction to potato production, Pudoc. Wageningen.
- Britto, D.T., Kronzucker, H.J., 2002. NH₄⁺ toxicity in higher plants: A critical review. *Journal of Plant Physiology* 159, 567–584. doi:10.1078/0176-1617-0774
- Cáceres, A., 2012. Manual de Laboratorio de Suelos y Aguas - Rangos de interpretación de análisis físicos y químicos de suelos. Facultad de Ciencias Agrícolas, Pecuarias Forestales y Veterinarias “Martín Cárdenas” - Universidad Mayor de San Simón, Cochabamba.
- Carballo, T., Gil, M. V., Calvo, L.F., Morán, A., Carballo, T., Gil, M. V., Calvo, L.F., Morán, A., 2009. The influence of aeration system, temperature and compost origin on the phytotoxicity of compost tea. *Compost Science & Utilization* 17, 127–139. doi:10.1080/1065657X.2009.10702411
- Chai, R., Ye, X., Ma, C., Wang, Q., Tu, R., Zhang, L., Gao, H., 2019. Greenhouse gas emissions from synthetic nitrogen manufacture and fertilization for main upland crops in China. *Carbon Balance and Management* 14, 1–10. doi:10.1186/s13021-019-0133-9
- Chodak, M., Khanna, P., Beese, F., 2003. Hot water extractable C and N in relation to microbiological properties of soils under beech forests. *Biology and Fertility of Soils* 39, 123–130. doi:10.1007/s00374-003-0688-0
- Condori, B., Devaux, A., Mamani, P., 1997. Efecto residual de la fertilización del cultivo de papa sobre el cultivo de haba (*Vicia faba* L.) en el sistema de rotación. *Revista Latinoamericana de la Papa* 9, 171–187.
- Cristobal-Alejo, J., Mora-Aguilera, G., Manzanilla-López, R.H., Marbán-Mendoza, N., Sánchez-García, P., Cid del Prado-Vera, I., Evans, K., 2006. Epidemiology and integrated control of *Nacobbus aberrans* on tomato in Mexico. *Nematology* 8, 727–737. doi:10.1163/156854106778877929
- Curtin, D., Beare, M.H., Qiu, W., 2021. Distinguishing functional pools of soil organic matter based on solubility in hot water. *Soil Research* 59, 319–328. doi:10.1071/SR20177
- Darby, H.M., Stone, A.G., Dick, R.P., 2006. Compost and manure mediated impacts on soilborne pathogens and soil quality. *Soil Science Society of America Journal* 70, 347–358. doi:10.2136/sssaj2004.0265
- de Guardia, A., Petiot, C., Rogeau, D., Druilhe, C., 2008. Influence of aeration rate on nitrogen dynamics during composting. *Waste Management* 28, 575–587. doi:10.1016/j.wasman.2007.02.007
- Deng, W., Zhang, A., Chen, S., He, X., Jin, L., Yu, X., Yang, S., Li, B., Fan, L., Ji, L., Pan, X., Zou, L., 2020. Heavy metals, antibiotics and nutrients affect the bacterial community and resistance genes in chicken manure composting and fertilized soil. *Journal of Environmental Management* 257, 109980. doi:10.1016/j.jenvman.2019.109980
- Diaz, L.F., Savage, G.M., Eggerth, L.L., Golueke, C.G., 1993. Composting and Recycling Municipal Solid Waste. California, USA.
- Drózdź, D., Wystalska, K., Malińska, K., Grosser, A., Grobelak, A., Kacprzak, M., 2020. Management of poultry manure in Poland – Current state and future perspectives. *Journal of Environmental Management* 264. doi:10.1016/j.jenvman.2020.110327
- Duong, T., 2013. Compost effects on soil properties and plant growth.
- Eguívar, R.D., Garcia, W., 2006. Alternativa agroecológica para el control del tizón tardío, *Phytophthora infestans*, de la papa en Colomi - Bolivia. *Acta Nova* 3, 564–577.
- Ellis-Jones, J., Mason, T., 1999. Livelihood strategies and assets of small farmers in the evaluation of soil and water management practices in the temperate inter-andean valleys of Bolivia. *Mountain Research and Development* 19, 221–234.
- Faissal, A., Ouazzani, N., Parrado, J.R., Dary, M., Manyani, H., 2017. Impact of fertilization by natural manure on the microbial quality of soil : Molecular approach. *Saudi Journal of Biological Sciences* 24, 1437–1443. doi:10.1016/j.sjbs.2017.01.005

- FAO, 1999. Bolivia hacia una estrategia de fertilizantes, in: Informe Preparado Para El Gobierno de Bolivia, Por El Proyecto Manejo de Suelos y Nutrición Vegetal En Sistemas de Cultivos GCPF/BOL/018/NET – “Fertisuelos.” p. 41.
- Fogarty, A.M., Tuovinen, O.H., 1991. Microbiological degradation of pesticides in yard waste composting. *Microbiological Reviews* 55, 225–233. doi:10.1128/mmr.55.2.225-233.1991
- Fox, J., Weisberg, S., Price, B., Adler, D., Bates, D., Baud-bovy, G., Bolker, B., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., Graves, S., Heiberger, R., Laboissiere, R., Maechler, M., Monette, G., Murdoch, D., Ogle, D., Ripley, B., Venables, W., Walker, S., Winsemius, D., Zeileis, A., R-core, 2018. Companion to Applied Regression.
- Franco, J., Ramos, J., Oros, R., 1999. Pérdidas económicas causadas por *Nacobbus aberrans* y *Globodera* spp . en el cultivo de la papa en Bolivia. *Revista Latinoamericana de la Papa* 11, 40–66.
- Gajbhiye, N.D., 2013. Toxic effect of sodium nitrate on germinating seeds of *Vigna radiata*. *International Journal of Biotechnology and Bioengineering* 7, 166–170.
- Gao, M., Liang, F., Yu, A., Li, B., Yang, L., 2010. Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere* 78, 614–619. doi:10.1016/j.chemosphere.2009.10.056
- Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry* 35, 1231–1243. doi:10.1016/S0038-0717(03)00186-X
- Godfray, C.H., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food Security : The Challenge of feeding 9 billion people. *Science* 327, 812–819. doi:10.1126/science.1185383
- Hanyabui, E., Samuel, O.A., Selorm, A., Joseph, S., 2021. Implementation of COVID-19 control measures: Effect on fertilizer supply and soil fertility in Africa. *African Journal of Agricultural Research* 17, 932–938. doi:10.5897/ajar2021.15621
- Hao, X., Chang, C., 2001. Gaseous NO, NO₂, and NH₃ loss during cattle feedlot manure composting. *Phyton - Annales Rei Botanicae* 41, 81–93.
- Headey, D., Fan, S., 2008. Anatomy of a crisis: The causes and consequences of surging food prices. *Agricultural Economics* 39, 375–391. doi:10.1111/j.1574-0862.2008.00345.x
- Hoover, N.L., Law, J.Y., Long, L.A.M., Kanwar, R.S., Soupir, M.L., 2019. Long-term impact of poultry manure on crop yield, soil and water quality, and crop revenue. *Journal of Environmental Management* 252, 109582. doi:10.1016/j.jenvman.2019.109582
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., 2017. Simultaneous inference in general parametric models.
- Iglesias Jiménez, E., Perez Garcia, V., 1989. Evaluation of city refuse compost maturity: a review. *Biological Wastes* 27, 115–142. doi:10.1016/0269-7483(89)90039-6
- INE, 2015. Censo agropecuario 2013 Bolivia. La Paz.
- Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil -V: A method for measuring soil biomass. *Soil Biology and Biochemistry* 8, 209–213. doi:10.1016/0038-0717(76)90005-5
- Khabarov, N., Obersteiner, M., 2017. Global Phosphorus Fertilizer Market and National Policies: A Case Study Revisiting the 2008 Price Peak. *Frontiers in Nutrition* 4, 1–8. doi:10.3389/fnut.2017.00022
- Khalil, A.I., Hassouna, S., 2013. Evaluation of the composting process through the changes in physical, chemical, microbial and enzymatic parameters. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 15, 25–42.
- Kollnig, S., 2020. Chicken for everyone? A cultural political economy of the popularity of chicken meat in Bolivia. *Gastronomica* 20, 36–48. doi:https://doi.org/10.1525/gfc.2020.20.4.36
- Kyakuwaire, M., Olupot, G., Amoding, A., Nkedi-Kizza, P., Basamba, T.A., 2019. How safe is chicken litter for land application as an organic fertilizer? A review. *International Journal of Environmental Research and Public Health* 16. doi:10.3390/ijerph16193521
- Lenth, R. V., 2016. Least-squares means: The R package lsmeans. *Journal of Statistical Software* 69. doi:10.18637/jss.v069.i01
- Li-Xian, Y., Guo-Liang, L., Shi-Hua, T., Gavin, S., Zhao-Huan, H., 2007. Salinity of animal manure and potential risk of secondary soil salinization through successive manure application. *Science of the Total Environment* 383, 106–114. doi:10.1016/j.scitotenv.2007.05.027
- Liang, R., Hou, R., Li, J., Lyu, Y., Hang, S., Gong, H., Ouyang, Z., 2020. Effects of different fertilizers on rhizosphere bacterial communities of winter wheat in the north China plain. *Agronomy* 10, 1–12.
- Lierop, W., Tran, T.S., Banville, G., Morissette, S., 1982. Effect of liming on potato yields as related to soil pH, Al, Mn, and Ca. *Agronomy Journal* 74, 1050–1055. doi:10.2134/agronj1982.00021962007400060028x
- Liu, K., Du, J., Zhong, Y., Shen, Z., Yu, X., 2021. The response of potato tuber yield, nitrogen uptake, soil nitrate nitrogen to different nitrogen rates in red soil. *Scientific Reports* 11, 1–10. doi:10.1038/s41598-021-02086-5
- Mann, E.N., Resende, P.M. de, Mann, R.S., Carvalho, J.G. de, Von Pinho, É.V. de R., 2002. Efeito da aplicação de manganês no rendimento e na qualidade de sementes de soja. *Pesquisa Agropecuária Brasileira* 37, 1757–1764. doi:10.1590/s0100-204x2002001200012

- Mondo, V.H.V., Guilhien, F., Junior, G., Leite, T., Pinto, F., Marchi, J.L. De, Viegas, A., Motomiya, D.A., Molin, J.P., Cicero, S.M., 2012. Spatial variability of soil fertility and its relationship with seed physiological potential in a soybean production area. *Revista Brasileira de Sementes* 34, 193–201.
- Motesharezadeh, B., Etesami, H., Bagheri-Novair, S., Amirmokri, H., 2017. Fertilizer consumption trend in developing countries vs. developed countries. *Environmental Monitoring and Assessment* 189. doi:10.1007/s10661-017-5812-y
- Nada, W.M., 2015. Stability and maturity of maize stalks compost as affected by aeration rate, C:N ratio and moisture content. *Journal of Soil Sciences and Plant Nutrition* 15, 751–764.
- Ndambi, O.A., Pelster, D.E., Owino, J.O., de Buissonjé, F., Vellinga, T., 2019. Manure Management Practices and Policies in Sub-Saharan Africa: Implications on Manure Quality as a Fertilizer. *Frontiers in Sustainable Food Systems* 3, 1–14. doi:10.3389/fsufs.2019.00029
- Pestalozzi, H., 2000. Sectoral fallow systems and management of soil fertility: The rationality of indigenous knowledge in the High Andes of Bolivia. *Mountain Research and Development* 20, 64–71. doi:10.1659/0276-4741(2000)020[0064:SFSATM]2.0.CO;2
- Peters, G., 2018. _userfriendlyscience: Quantitative analysis made accessible_. doi:doi: 10.17605/osf.io/txequ
- Poll, J., Marhan, S., Haase, S., Hallmann, J., Kandeler, E., Russ, L., 2007. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. *FEMS Microbiology Ecology* 62, 268–279. doi:10.1111/j.1574-6941.2007.00383.x
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raut, M.P., Prince William, S.P.M., Bhattacharyya, J.K., Chakrabarti, T., Devotta, S., 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste - A compost maturity analysis perspective. *Bioresource Technology* 99, 6512–6519. doi:10.1016/j.biortech.2007.11.030
- Riffaldi, R., Levi-Minzi, R., Pera, A., de Bertoldi, M., 1986. Evaluation of compost maturity by means of chemical and microbial analyses. *Waste Management & Research* 4, 387–396.
- Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nadelhoffer, K.J., Harris, D., 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potential, in: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, New York, USA, pp. 258–271.
- Rodríguez-Kábana, R., 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology* 18, 129–135.
- Rynk, : Robert, 1992. *On-Farm Composting Handbook*. Monographs of the Society for Research in Child Development 77, 132.
- Savci, S., 2012. Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia* 1, 287–292. doi:10.1016/j.apcbee.2012.03.047
- Soremi, A., Adetunji, M., Adejuyigbe, C., Bodunde, J., Azeez, J., 2017. Effects of poultry manure on some soil chemical properties and nutrient bioavailability to soybean. *Journal of Agriculture and Ecology Research International* 11, 1–10. doi:10.9734/jaeri/2017/32419
- Spiess, A.-N., 2018. Propagation of uncertainty using higher-order Taylor expansion and Monte Carlo simulation.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418, 671–677.
- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. *Science* 292, 281–284. doi:10.1126/science.1057544
- Vargas, M., 2017. Apuntes sobre la agricultura familiar en Bolivia. Instituto Interamericano de Cooperación para la Agricultura, La Paz.
- Westwood, J.H., Foy, C.L., 1999. Influence of nitrogen on germination and early development of broomrape (*Orobancha* spp.). *Weed Science* 47, 2–7. doi:10.1017/s0043174500090573
- Wickham, H., Romain, F., Lionel, H., Müller, K., 2019. dplyr: A Grammar of data manipulation.
- Willson, G.B., Dalmat, D., 1986. Measuring Compost Stability. *BioCycle* 27, 34–37.
- Wood, S.N., 2017. Generalized Additive Models: An Introduction with R (2nd Edition). *Journal of Statistical Software* 86, 1–5. doi:10.18637/jss.v086.b01
- Xiao, Z., Zou, T., Lu, S., Xu, Z., 2020. Soil microorganisms interacting with residue-derived allelochemicals effects on seed germination. *Saudi Journal of Biological Sciences* 27, 1057–1065. doi:10.1016/j.sjbs.2020.01.013
- Yang, Q., Ren, S., Niu, T., Guo, Y., Qi, S., Han, X., Liu, D., Pan, F., 2014. Distribution of antibiotic-resistant bacteria in chicken manure and manure-fertilized vegetables. *Environmental Science and Pollution Research* 21, 1231–1241. doi:10.1007/s11356-013-1994-1
- Zimmerer, K.S., 1993. Soil erosion and social (Dis) courses in Cochabamba, Bolivia: perceiving the nature of environmental degradation. *Economic Geography* 69, 312–327. doi:10.2307/143453
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. *Mixed effects models and extensions in Ecology with R*. Springer Science, New York.