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SHORT-TERM EFFECTS OF SOIL AMENDMENT WITH DAIRY SLUDGE ON YIELD, BOTANICAL COMPOSITION, MINERAL NUTRITION AND ARBUSCULAR MYCORRHIZATION IN A MIXED SWARD

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SUMMARY

A field study was carried out to evaluate the short-term effects of increasing dairy sludge application rates on yield, botanical composition and nutrient content of the first regrowth of a grass-white clover sward. Arbuscular mycorrhizal (AM) root colonization and forage phosphorus concentration were also analyzed five months after dairy sludge addition. The cultivated soil was a Humic Cambisol which was limed, NPK-fertilized and sown with a mixture of perennial ryegrass, cocksfoot and white clover. After a silage cut made in June 1997, four dairy sludge rates were applied: 0, 80, 160 and 240 m^3 ha^{-1}.

Increased rates of the sludge resulted in higher DM yields of the first regrowth. Soil fertilization with 240 m^3 ha^{-1} sludge favoured grass growth and competition, while decreased clover proportion (6.5%) in the sward. Higher percentages of clover (20%) were found when 80 or 160 m^3 ha^{-1} of dairy sludge were applied. Concentrations of N, P and K were greater in forage from sludge-added plots than in the control. There were no differences between sludge treatments. AM root colonization slightly decreased on adding any of the sludge rates to the sward compared with control, which was related to higher P concentration in forage. Results indicated that optimal dose was 80 m^3 ha^{-1}, which yielded only 30% less than the highest sludge rate and best favoured competition of clover against grass.

Key words: Forage production, grass/white clover swards, soil indigenous AM fungi, organic fertilization, organic wastes recycling.

INTRODUCTION

Most dairy industries produce sludges from a biological treatment of milk residues and cleaning waters, which have significant amounts of organic matter and mineral nutrients (particularly N and P) and a low heavy metal content (De Lauzanne and

Merillot, 1986; López-Mosquera *et al.*, 1999). Agricultural use can be a suitable and cost-effective way to incorporate these wastes into the matter and energy cycles.

In milk-producing areas, where those industries are established, farms produce high amounts of slurry which is currently applied as fertilizer on mixed swards. In many farms, slurries are insufficient to fertilize the whole grassland surface, other waste materials or mineral fertilizers also being used. In fact, dairy sludge has been applied in grasslands in Galicia for the last seven years, although there is no information about the optimal application rates for forage production. The availability of nutrients, particularly N, in the short-term is one of the factors to be considered in recommending dairy sludge application rates after forage cutting.

Application of these wastes in sustainable agronomic systems must also take into account their impact on arbuscular mycorrhizas (AM), a mutualistic symbiosis between certain Glomalean fungi and the roots of most cultivated plants, which may play a relevant role in nutrient cycling and plant growth in grassland soils amended with organic materials. Many works have shown that AM fungi can increase plant growth and P and other elements uptake (Hayman, 1983; Marschner and Dell, 1994). Agronomic practices, such as fertilizer application, may have detrimental effects on formation and survival of soil AM fungal populations and root colonization (Thompson, 1994). Some little information exists on the effects of urban wastes on the symbiosis. Lambert and Weidensaul (1991) found a negative effect of sewage sludge application on the mycorrhizal colonization of soybean. Recently, Sainz *et al.* (1998) found a decrease in AM root colonization of red clover on increasing the proportion of a composted urban refuse in the growth substrate. However, no research has been done on the impact of dairy sludge application on AM symbiosis in mixed swards.

The aim of this work was to evaluate, in the short-term, the effects of several dairy sludge application rates on yield, botanical composition and mineral nutrition of the first regrowth of a mixed sward. Also, AM root colonization was studied in grass and clover roots five months after dairy sludge application to sward and related to forage P concentration.

MATERIAL AND METHODS

The experiment was carried out at Goiriz (Lugo, Spain, 43° 17' 28.5" N, 7° 38' 27.3" W) in a mixed sward sown in autumn 1996. Mean annual rainfall in the area is 1176 mm, average maximum temperature is 16.8 °C and minimum average temperature 6.2 °C. The soil is a Humic Cambisol (FAO, 1991) characterised by a sandy-loam texture, a pH

(H_2O) of 5.4, a high level of organic matter (7.1%) and a low effective exchange capacity (5.66 cmol kg^{-1}). It was limed with 3 t of dolomitic limestone per ha, to neutralize exchangeable aluminium, and received 600 kg ha^{-1} of a 8:24:16 NPK fertilizer before sowing the following seed mixture: *Lolium perenne* L. cv 'Barvestra' 20 kg ha^{-1}, *Dactylis glomerata* L. cv 'Nicol' 10 kg and *Trifolium repens* L. cv 'Huia' 3 kg. In spring 1997, 250 kg ha^{-1} of 9:18:27 NPK fertilizer were applied.

Before starting the experiment, forage was cut for silage in June 1997. Inmediately after the cut, twelve 30 x 10 m^2 plots were selected on the sward on a uniform site with a slope under 2%, and four rates of dairy sludge were applied: 0, 80, 160 and 240 m^3 ha^{-1}. Treatments were randomly applied, using three replicates per treatment. The dairy sludge was provided by Lactalis-Leche de Galicia, a milk producing company, and its main characteristics are given in Table 1. A uniform, low-trajectory application of the sludge to each plot area was achieved by using a tank which was tractor-drawn. Amounts of N, P, K, Ca and Mg provided by the sludge at different application rates are presented in Table 2.

Forage yield was estimated after one and a half month regrowth by harvesting six 33 cm x 33 cm quadrats, thrown randomly in each plot, to a height of 3 cm above ground level. The cut was made when the sward in the experimental plots was 25 cm height, as measured with a graduated ruler, by using battery-operated hand-held clippers. The remaining forage in each experimental plot was cut with a Bertollini mower to the same stubble height as in quadrats to maintain uniform forage conditions. Herbage from each quadrat was hand-separated into grass, white clover and weed fractions. All three fractions were dried to constant weight for 48 h at 65 °C to estimate total dry-matter yield per ha and the proportion of grasses, clover and weeds of the forage. Then, the three dried fractions of each quadrat were mixed up and a representative sample of forage taken for nutrient analysis. After grinding, 100 mg of forage were digested with concentrate H_2SO_4 and 30% H_2O_2 (Thomas *et al.*, 1967) for analysis of shoot N, P, K, Ca and Mg contents. Nitrogen was determined by the Kjeldahl method (Cottenie, 1980), phosphorus by UV/V spectrometry (Dick and Tabatabai, 1977) and K, Ca and Mg by atomic emission/absorption spectroscopy.

After the cut made in July, cattle was allowed to graze the sward until October. On 4th November 1997, at sites free from dung deposition, ten soil samples containing roots were randomly taken at 0-15 cm depth along a longitudinal transect in each plot, using a manually driven corer, in order to study the effect of dairy sludge application on AM root colonization. The corer had an inner cutting diameter of 7 cm. Roots were separated from soil and gently washed with tap water. Only roots from grasses and clover were then selected and kept separately in 70% ethanol until analysis of root colonization by indigenous AM fungi. Roots were cleared and stained with trypan blue (Phillips and Hayman, 1970) and the percentage of AM root colonization estimated by the gridline intersect method (Ambler and Young, 1977), counting 200 intersection points per root sample (Giovannetti and Mosse, 1980). Since mycorrhizal colonization has been extensively related to plant P concentration (Menge *et al.*, 1978; Sainz and Arines, 1988), a forage sample was also taken in the location of each soil sample to analyze P.

All data were subjected to a one-way ANOVA, using the LSD test for means separation, with the SPSS statistical software.

RESULTS

Plant growth and botanical composition

Increasing dairy sludge rates increased forage production. With the highest application rate, 5410 kg DM ha^{-1} were obtained, which represents an increased yield of 143% with respect to control and 30% with respect to both other sludge rates studied (Figure 1). No significant differences in forage DM production were found between 80 and 160 m^3 ha^{-1} sludge treatments.

Botanical composition of forage significantly varied among treatments (Figure 2). The presence of grass in sward was favoured by the addition of 160 m^3 ha^{-1} of dairy sludge and especially of 240 m^3 ha^{-1}, this last rate resulting in 90% grass forage.

A low percentage of white clover was recorded in control plots. Presence of clover in forage increased on increasing dairy sludge rate up to 160 m^3 ha^{-1}. The percentage of clover in plots receiving 240 m^3 ha^{-1} was only 6.5, a value similar to that found in controls (4%).

Control plots presented the highest proportion of weeds (42%), which were significantly decreased by dairy sludge addition to percentages under 5%.

Nutrient concentration and content in the forage

One and a half month after dairy sludge application, N, P and K percentages in forage were significantly increased in fertilized plots respect to the control, although no differences were found among sludge treatments (Table 3). The addition of dairy sludge had no effect on Ca and Mg percentages in shoots (Table 3).

Results on N, P, K, Ca and Mg uptake in shoots followed the same trends as DM production values (Table 4). Highest nutrient contents in forage were found when sward received 240 m^3 ha^{-1} of dairy sludge, no differences being observed between 80 and 160 m^3 ha^{-1} application rates.

AM root colonization and forage P concentration

Percentages of root colonization by AM indigenous fungi were high both for grass and white clover in all treatments (Figure 3). Highest percentages were observed in control treatment with respect to sludge-fertilized plots, which showed no differences among them.

Roots from grass and white clover presented similar percentages of AM root colonization within each dairy sludge treatment.

In November, as observed in July, P concentration in forage also increased on increasing dairy sludge application rate in relation to the control (Figure 3), only that P values were higher.

DISCUSSION

Increasing application rates of dairy sludge to swards significantly increased forage yields of the first regrowth (Figure 1). Similarly, enhanced plant production has been reported when recycling municipal sludges in agricultural systems with different crops (Bevacqua and Mellano, 1994; Hue and Subasinghe, 1994; Misselbrook *et al.*, 1996). Increased yields have been frequently related to nutrient supply derived from sewage sludge application, mainly to N and P. Edgar *et al.* (1995), in a field study in which a liquid anaerobically digested sewage sludge was applied to grassland, concluded that the sludge was a useful nitrogenous manure when applied in late winter/early spring. More recently, Misselbrook *et al.* (1996) carried out sewage sludge applications to grassland in autumn, winter and spring (following first silage cut) and found a yield benefit from spring rather than autumn injection of raw sludge when wet conditions occurred prior to and following spring injection. Authors related these results to an increased crop N. We also found a significant increase in forage N (Table 3) as a result of dairy sludge application, although no differences were observed among the three sludge treatments. Soil fertilization with the highest dairy sludge rate benefited grass growth and competition and decreased clover proportion, forage presenting 90% grass (Figure 2). At intermediate dairy sludge doses (80 and 160 m^3 ha^{-1}), a better grass-legume ratio was found with respect to the highest dose treatment, white clover representing around 20% forage composition. Skousen and Clinger (1993) also found an increased biomass of forage in a minesoil as amended with increasing doses of sewage sludge, which was related to a grass biomass increase and a legume biomass decrease. The higher clover proportion found in our work for intermediate dairy sludge application rates probably explain similar N percentages in forage (and consequently similar protein contents) as in the highest sludge rate treatment. This result further confirms the major role played by legumes in sustainable grassland systems.

While the effect of dairy sludge-derived nitrogen on forage production and botanical composition seems to be beyond doubt, it can not be discarded a relevant role of P and water. The highest dairy sludge application rate supplied the soil with 84 kg P ha^{-1} (Table 2) and the lowest one with 28 kg P ha^{-1}. In correspondence, forage P concentration significantly increased on increasing dairy sludge rate, both in July and November samplings. Brown *et al.* (1990) also reported an increased yield of tall fescue in plots fertilized with lime-stabilized sludge from a dairy wastewater treatment, which was added as a source of lime and P.

Our results on forage P concentration can be related to AM root colonization of grass and clover by indigenous soil fungi (Figure 3). In spite of only five months having passed since dairy sludge application, a slight decrease in AM colonization of grass and clover roots was observed in sludge-added plots respect to control. Many mycorrhizal studies have reported a decreased AM root colonization on applying increasing rates of phosphate fertilizers (Sainz and Arines, 1988; Marschner and Bell, 1994) and also, as found in our work, on increasing plant P concentration (Menge *et al.*, 1978). To this respect, Weissenhorn *et al.* (1995), in a field study carried out in an acid sandy soil receiving sewage sludge for several years, also reported a decrease in the percentage of AM maize root colonization, which was related to higher percentages of plant P respect to control, while no relationship between mycorrhizal abundance and degree of metal exposure in soil or inside plant roots were observed.

Another aspect which might have been relevant to achieve increased forage production in our work is the amount of water supplied by dairy sludge. For instance, the highest application rate supplied the soil with approximately 200 m^3 water per ha. In the area where our research was done, a period of drought is commonly observed from July to September, irrigation being not applied to grasslands which suffer an important limitation for forage production in the summer. The significant amount of water, together with mineral nutrients, supplied by dairy sludge application clearly accounted for yields observed after first regrowth.

In conclusion, our results indicate that best application rate of dairy sludge, for sward sustainability, was 80 m^3 ha^{-1}, which yielded 30% less than the highest sludge rate and only slightly decreased AM root colonization. Also in respect to this highest application rate, addition of 80 m^3 ha^{-1} favoured competition of clover against grass, the legume representing around 20% forage. This clover percentage was related to forage N and P concentrations comparable with those achieved with higher sludge application rates.

Although previous research carried out by our group showed low heavy metal content in dairy sludge (López Mosquera *et al.*, 1999), field application of moderate sludge rates could best prevent a possible accumulation of those metals in the long-term in agricultural systems, considering that cultivated fields also receive metals from other organic and mineral fertilizers.

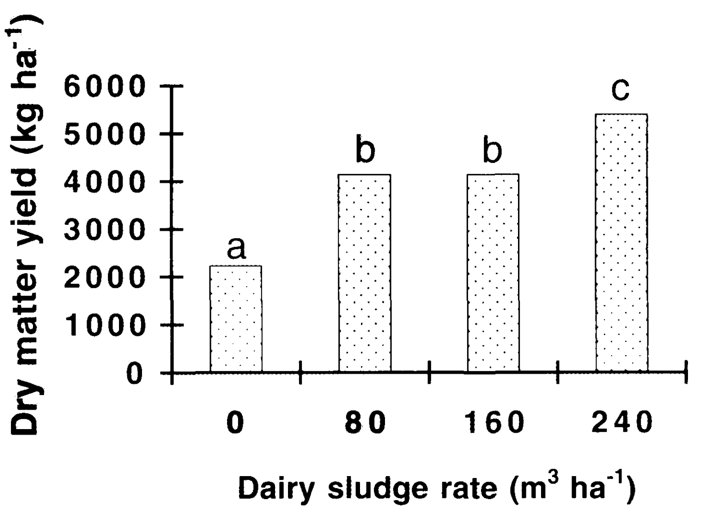

FIGURE 1

Forage yield (kg ha^{-1}) in the first regrowth of the sward from control and fertilized plots one and a half months after dairy sludge application. Values represented by bars with the same letter are not significantly different at $p \leq 0.05$.

Producción de forraje (kg ha^{-1}) en el primer rebrote del prado en las parcelas control y en las fertilizadas mes y medio después de la aplicación del lodo de industria láctea. Los valores representados por barras con la misma letra no son significativamente diferentes para $p \leq 0,05$.

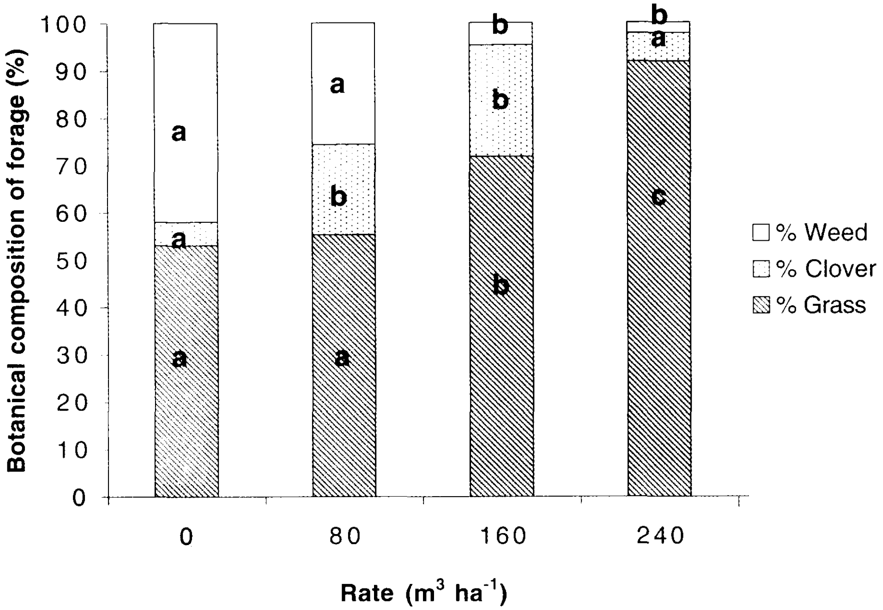

FIGURE 2

Grass, white clover and weed proportions in the first regrowth of the sward from control and fertilized plots one and a half months after dairy sludge application. Values represented by bars with the same letter are not significantly different at $p \leq 0.05$.

Proporción de gramíneas, trébol blanco y malas hierbas en el primer rebrote del prado en las parcelas control y en las fertilizadas mes y medio después de la aplicación del lodo de industria láctea. Los valores representados por barras con la misma letra no son significativamente diferentes para $p \leq 0{,}05$.

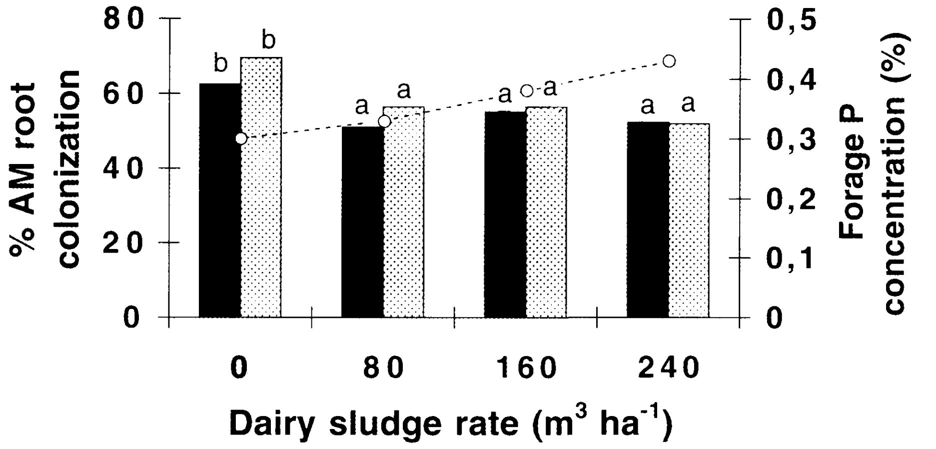

FIGURE 3.

Forage P concentration (O) and percentage of root length colonized by soil indigenous AM fungi in ■ grass and □ white clover from control and dairy sludge-fertilized plots, in November 1997. Values represented by bars for either grass or clover botanical fractions with the same letter are not significantly different at p≤ 0.05.

Concentración de P en el forraje (O) y porcentaje de longitud de raíz colonizada por hongos MA indígenas del suelo en ■ gramíneas y □ trébol blanco en las parcelas control y en las fertilizadas, en noviembre de 1997. Los valores representados por barras con la misma letra no son significativamente diferentes para p≤ 0,05.

TABLE 1

Mean values of parameters analysed in the dairy sludge used in the experiment (dry wt 13 g L^{-1})

Valores medios de los parámetros analizados en el lodo de industria láctea utilizado en el ensayo (extracto seco de 13 gL^{-1})

General properties		Total heavy metals ($mg\ kg^{-1}$ dry wt)		E.U. heavy metal limits* ($mg\ kg^{-1}$ dry wt)
pH	7.40	Zn	180	2500
E.C. ($dS\ m^{-1}$)	2.35	Cr	14.5	1000
Total-N (% of dry wt)	6.90	Pb	22.2	750
Organic-C (% of dry wt)	38.30	Cu	50.3	1000
C/N	5.55	Ni	77.6	300
P (% dry wt)	2.69	Cd	<10	20
K (")	0.92			
Ca (")	2.00			
Mg (")	0.46			
Na (")	4.38			

* E.U. Directive 86/278/CEE. 12 june 1986

TABLE 2

Mineral inputs (kg ha^{-1}) of the dairy sludge at different application rates

Aportes minerales (kg ha^{-1}) del lodo de industria láctea a diferentes dosis

Rate of dairy sludge (m^3 ha^{-1})	N	P	K (kg ha^{-1})	Ca	Mg
80	71	28	9.6	20.8	4.8
160	140	56	19.2	41.6	5.6
240	213	84	28.8	62.4	14.4

TABLE 3

Concentrations (g kg^{-1}) of N, P, K, Ca and Mg in forage. Values followed by the same letter within a column are not significantly different at $p \leq 0.05$

Concentraciones (g kg^{-1}) de N, P, K, Ca y Mg en el forraje. Los valores seguidos por la misma letra dentro de cada columna no son significativamente diferentes para $p \leq 0{,}05$

Rate of dairy sludge (m^3 ha^{-1})	N	P	K (g kg^{-1})	Ca	Mg
0	15.7 a	2.0 a	24.8 a	3.7 a	1.9 a
80	23.9 b	2.2 ab	28.6 b	4.1 a	1.9 a
160	24.9 b	2.5 b	32.0 b	3.9 a	2.1 a
240	26.2 b	2.6 b	30.7 b	3.6 a	2.2 a

TABLE 4

Nitrogen, P, K, Ca and Mg uptake (kg ha^{-1}). Values followed by the same letter within a column are not significantly different at $p \leq 0.05$

Extracciones de N, P, K, Ca y Mg en kg ha^{-1}. Los valores seguidos por la misma letra dentro de cada columna no son significativamente diferentes para $p \leq 0{,}05$

Rate of dairy sludge (m^3 ha^{-1})	N	P	K (kg ha^{-1})	Ca	Mg
0	34.92 a	10.67 a	55.15 a	8.23 a	4.22 a
80	98.92 b	21.52 b	118.37 b	16.97 b	7.86 b
160	103.36 b	24.07 b	132.83 b	16.19 b	8.72 b
240	141.71 c	32.99 c	166.05 c	19.47 c	11.9 c

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EFECTOS A CORTO PLAZO DE LA ENMIENDA DEL SUELO CON LODOS DE INDUSTRIA LÁCTEA SOBRE LA PRODUCCIÓN, COMPOSICIÓN BOTÁNICA, NUTRICIÓN MINERAL Y MICORRIZACIÓN ARBUSCULAR EN PRADERAS MIXTAS.

RESUMEN

Se llevó a campo un experimento de campo para evaluar los efectos a corto plazo de la aplicación de dosis crecientes de lodo de industria láctea sobre la producción, composición botánica y contenido en nutrientes del primer rebrote de una pradera mixta. Cinco meses después de la aplicación del lodo, se analizaron también la colonización de la raiz por hongos formadores de micorrizas arbusculares (MA) y la concentración de P

en el forraje. El suelo de cultivo es un Cambisol Húmico que se había encalado, abonado con un fertilizante NPK y sembrado con una mezcla de raigrás inglés, dactilo y trébol blanco. Después del corte de silo realizado en junio de 1997, se aplicaron cuatro dosis del lodo: 0, 80, 160 and 240 m^3 ha^{-1}.

El incremento en la dosis de lodo determinó una mayor producción de materia seca en el primer rebrote. La dosis de 240 m^3 ha^{-1} favoreció el crecimiento y la competitividad de las gramíneas, mientras que redujo la proporción de trébol (6,5%) en el prado. Se obtuvieron porcentajes más altos de trébol (20%) con las dosis de 80 y 160 m^3 ha^{-1}. Las concentraciones de N, P y K del forraje fueron más altas en las parcelas en las que se añadió el lodo que en el control. No hubo diferencias entre los tratamientos de lodo. Respecto al control, la aplicación de cualquiera de las dosis de lodo al prado causó una ligera disminución de la colonización MA de la raiz, lo cual se relacionó con una mayor concentración de P en el forraje. Los resultados indicaron que la dosis óptima había sido la de 80 m^3 ha^{-1}, ya que con ella se obtuvo una producción de forraje inferior en solo un 30% a la obtenida con la dosis más alta de lodo y favoreció mejor que ésta la competitividad del trébol frente a las gramíneas.

Palabras clave: Producción de forraje, praderas de gramíneas/trébol blanco, hongos MA indígenas del suelo, fertilización orgánica, reciclado de residuos orgánicos.