Digestate application on two different soils: agricultural benefit and risk

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Abstract

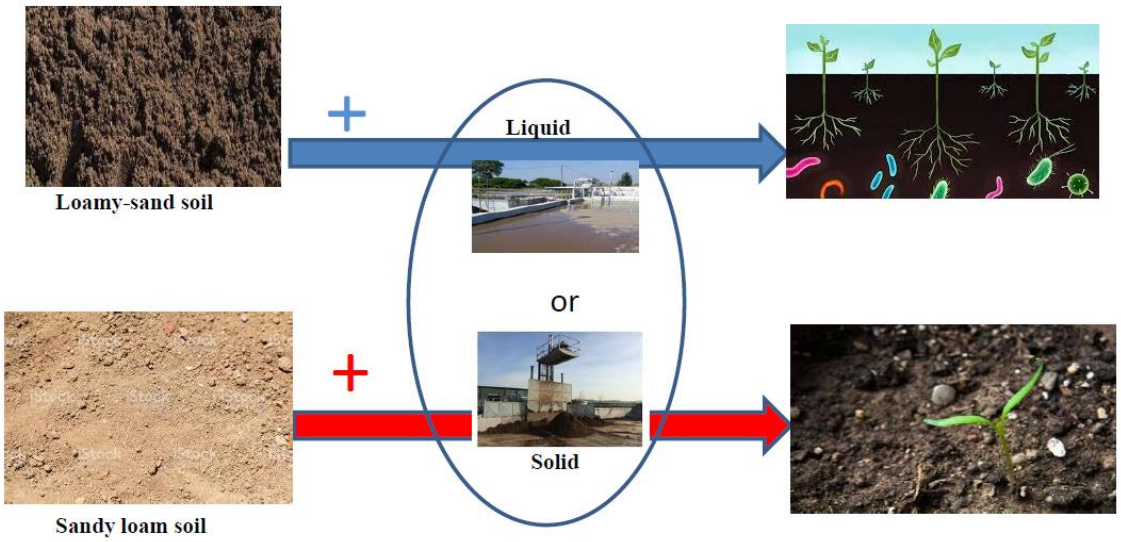
In order to valorize the use of digestate, the purposes of this study was to discriminate the fertilizing potential of solid and liquid fractions of digestate using two soils that differed for chemical characteristics, to expand the digestate use reducing its environmental impact. The two fractions did not contain toxic compounds and differed in chemical compositions. The two soils responded differently to the addition of the two-digestate fractions and the benefit depended mainly on soil characteristics rather than on quantity and quality of the organic material applied. In the soil with neutral pH, the highest intrinsic amount of organic matter,

microbial biomass (MBC), fungi, bacteria and cation exchange capacity were observed; all these properties increased the most over time, in presence of both solid and liquid fractions. Differently, in the soil with alkaline pH and minor amount of intrinsic organic matter, MBC, fungi and bacteria, only few properties such as oxidative soil activity, bacteria colonies, and organic matter amount were improved by the addition of digestate fractions. The use of both fractions showed more agricultural advantages in respect to the relative risks, and the solid fraction was the most effective. Even if the effects of digestate on soil ecosystem can differ in extent, we can expect economic benefit deriving from the reduction of the costs for its disposal, agricultural benefit for their high supply of nutrients to the soil and environmental advantages for the decrement in the use of manufactured fertilizers.

Keywords: digestate fraction, microbial activity, organic matter, soil fertility, soil properties

Shortcut list: CEC (cation exchange capacity); **C/N** (carbon nitrogen ratio), **DHA** (dehydrogenase); **DL** (liquid fraction of digestate); **DS** (solid fraction of digestate); **EC** (electric conductivity); **FDA** (fluorescein 3,6-diacetate hydrolase); MBC (microbial biomass C); **OC** (organic carbon); **OM** (Organic matter); **OP (**osmolarity); **RIZ** (loamy-sand soil)**;** **SANL** (sandy-loam soil); **WSP** (water soluble phenols).

Graphic Abstract



Statement of Novelty

In order to expand the digestate use reducing its environmental impact, this study discriminate the fertilizing potential of solid and liquid fractions of digestate using two soils that differed for chemical characteristics. This study provides the indications in which the soil quality benefits from the use of liquid and solid digestate fractions as fertilizer. The level and nature of advantages depend mainly on soil characteristics rather than quantity (carbon loading) and quality (decomposability) of the organic material applied. Both fractions showed more agricultural advantages in respect to the relative risks, and the solid in comparison with the liquid fraction was the most effective.

Introduction

In the last years, investments and promotions in the development of renewable energy sources are growing, particularly in emerging markets, due to the increase in population, extensive

industrialization, limited fossil energy resources and climate change (Mathiesen et al., 2011). Additionally, in accordance with the Renewable Energy Directive (2009), all the EU member countries have to accomplish by 2030, through the achievement of individual national targets, a production of energy from renewable sources of at least 32% of own total energy. The third most important renewable energy source comes from biomasses. Biomass energy is the conversion of different kind of biomasses into useful forms of energy for heating, electricity generation and transport fuels. Biomass for bioenergy comes from dedicated energy crops (Braun et al., 2008), wastes generated in the agro-industrial processing (Srirangan et al., 2012; Reddy and Srinivas, 2013) and municipal wastes (Sortino et al., 2014; Tampio et al., 2016).

Anaerobic digestion of organic wastes is considered a key process to produce renewable energy able to meet the growing energy demand in a sustainable way. The focus of the anaerobic digestion plant is the optimization of the biological process in order to maximize the production of biogas. However, in order to achieve the sustainability of energy chain and the economic balance of the plant management, an increasingly important factor is the profitable reuse of its by-product, the digestate (Holm-Nielsen et al., 2009).

As shown by Möller and Müller (2012), Makádi et al. (2012), and Barłóg et al. (2020), the digestate produced by anaerobic digestion is a rich source of nitrogen (N), phosphorus (P), potassium (K), and sulphur (S), various micronutrients and organic matter, whose addition to the soils can help to stimulate microbial biomass and soil metabolic activities improving soil ecosystem functioning. Digestate affects N cycle resulting in an increase in the proportion of total N that is more readily plant available (i.e., in increase in the ratio of ammonium-N ($NH+_4$-N) to total N, about 70%) (Panuccio et al., 2016) and in a decrease in the C:N ratio (Möller et al., 2012; Tampio et al., 2016). Some studies evidenced that the digestate can act as "primer" increasing soil organic matter decomposition (Fontaine et al., 2003; Abubaker et al., 2013) with a gains of additional pool of mineral N readily available for plants (Mason-Jones

et al., 2018). Chantigny et al. (2008) demonstrated no differences in the risk of nitrate leaching from digested in comparison to undigested animal slurries. However, the application of digestate could cause risk of volatilization and/or dispersal of nitrogen forms, mainly if disposed on soil surface. Moller (2015) reported that the negative effects of digestate on long-term sustainability in terms of soil fertility and environmental impact at field level were in any case of minor relevance. The use of digestate as amendment could minimize the enormous demand for synthetic chemical fertilizers and consequently lower the economic and environmental costs associated with chemical fertilizer production, and with waste disposal (European Environment Agency, 2010). The characteristics of digestate depend on the nature and composition of the ingested material as well as the parameters settled in the biogas processes and, among the latter, the retention time plays a key role. The longer the retention time is, the lower is the residual organic matter amount. The retention time affects the methanogenesis (Szűcs et al., 2006) and, consequently, the chemical composition of digestate, which in turn defines its agronomic efficacy.

The first step in digestate processing is generally the separation of solid phase from the liquid phase for its easy management and use. The liquid digestate contains less than 15% dry matter (DM) content, while the solid digestate contains more than 15% DM. The solid fraction is generally directly used in agriculture for fertilizer purpose, instead, the liquid fraction, based on its composition, is employed for fertigation of crops, implementing fresh water saving program.

The effects of digestate as amendment have been extensively studied (Elbashier et al., 2018) and how change the fertilizing power of the application of the unfractionated digestate in respect to soil characteristics have been also object of researches. Makadi et al. (2016) demonstrated a different behavior of digestate when applied on soils belonging to different textural class, showing that sandy soils were more affected by digestate than loamy soils.

In order to further valorize the use of this by-product and make economically and environmentally sustainable the biogas production chain closing the process cycle, the purpose and the novelty of this study were to examine the effects of digestate separated in liquid and solid fractions on two soils differing for chemical and biological characteristics to verify if they were the chemical soil properties to influence liquid and solid fraction effectiveness differently, or it was the chemical composition of the single fractions to affect soil characteristics.

The soils (loamy-sand and sand-loamy) were selected because they were representative of most of the cultivated land in Calabria. The specific objective was to verify if both the fractions of digestate can be transversally used as fertilizer on different soil types or if their effects can be driven by soil characteristics.

Materials and Methods

Experiment and soil sampling

The soils used in this study were collected from two different locations in South Italy, Calabria. The soil collected from Rizziconi contrada Turbine (Italy, Calabria, 50º7′N, 14º22′E) was conventionally named **RIZ**, the second one collected from San Lorenzo, Reggio Calabria (Italy, Calabria, 50º7′N, 14º22′E), was named **SANL**. The soils were classified as Eutric-Cambisol, textural class loamy-sand (7% clay, 11% silt, and 84% sand) haplic Cambisol textural class sandy-loam (17% clay, 11% silt, and 72% sand), respectively, according to FAO (1999) system criteria. The main chemical and biological characteristics of these two soils are showed in Table 1.

The liquid and solid fractions of digestate used in these experiments were purchased from the biogas plant, Fattoria della Piana located in Candidoni (Calabria, Italy). This biogas plant is

the largest existing in the central and southern Italy with 998 kWel of installed power. The starting biomass consisted of animal manures (poultry, cow and sheep), milk serum, maize silage and in minor amount of olive waste and citrus pulp. The digestate fractions were analyzed as reported in Panuccio et al. (2016) and their chemical composition is shown in Tables 2 and 3.

Experimental set-up and design of soil amendment

The experiments were performed in plastic pots (19 cm diameter) filled with 3.5 kg of soil amended with the solid fraction (DS) of digestate used as fresh mass at the percentage of 25, 50 and 75% (w/w), or with the liquid fraction (DL) at the concentrations of 10, 25 and 50%. the doses of digestate were deliberately chosen up to high values to verify if their high doses negatively influenced the biological characteristics of both soils and to evaluate whether the effects on the soils were linear or Gaussian.

The experimental design consisted of six pots for each treatment and non-amended soil was used as control. The experiment was conducted in glass house to protect soil from rainfall, managing the irrigation system to maintain 70% of field capacity at a temperature of 25°C. Non-amended and amended soils were collected and analyzed 6 months after the treatments. Soil samples were homogenized and sieved (2 mm mesh), then were subdivided in two sub-samples, one of them was kept at 4 °C for biochemical assays until processing.

Physical and chemical Analysis

Chemical parameters were determined in three replicates. Particle size analysis was carried out by the hydrometer method, using sodium hexametaphosphate as a dispersant (Bouyoucos, 1962); dry matter content of un-amended and amended soils was determined at 105°C until the mass loss of the sample during 24 h was lower than 0.5% of its weight; pH was measured

in distilled water and 1 M KCl using a 1:2.5 (digestate or soil/water) suspension; electrical conductivity (EC) was determined in distilled water by using 1:5 digestate or soil:water suspension, after shaking at 15 rpm for 1 h to dissolve soluble salts, and then detected by Eutech instrument conductivity meter; organic carbon (OC) was determined by the Walkley–Black procedure based in the determination of the Cr^{3+} resulting from organic C oxidation (Walkley and Black, 1934), and it was converted to organic matter (OM) by multiplying the percentage of carbon by 1.72; total nitrogen (Ntot) was measured by Kjeldahl method (Kjeldahl, 1883). The C/N ratio was calculated as total organic carbon/ total nitrogen (TOC/Ntot).

Biochemical analysis

Microbial Biomass Carbon (MBC) was determined by the chloroform fumigation-extraction procedure (Vance et al., 1987) with soil moist samples (equivalent to 20 g d.s.). Soil samples were fumigated with alcohol-free $CHCl_3$ for 24 h at 24 °C. Both fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 (1:4 w/v) and filtered with Whatman's n^o 42 paper and then were analyzed for soluble organic C, using the method of Walkley and Black (1934). MBC was estimated based on the difference between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C (Vance et al., 1987). Microbial activity (FDA) was determined by the hydrolysis of fluorescein 3,6-diacetate into fluorescein, according to Adam and Duncan's protocol (Adam and Duncan, 2001). Briefly, 15 ml of 60 mM potassium phosphate pH 7.6 and 0.2 ml of 1000 μg FDA ml^{-1} were added to 2 g of fresh amended and un-amended soils, sieved < 2 mm. The flasks were then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added to terminate the reaction. The content of the flask

was centrifuged (Digicem 21 R, Ortoalresa Inc., Madrid, Spain) at 2000 rpm for 3 min. The supernatant was filtered through Whatman no 42. The optical density of clarified filtrates was determined at 490 nm (Shimadzu UV–Vis 2100, Japan). The enzyme activity was expressed in micrograms of fluorescein per gram of soil per hour. Dehydrogenase (DHA) activity was determined with the method of von Mersi and Schinner (1991): A sample of fresh soil equivalent to 1 g of oven dried (105 °C) soil was added with 1.5 ml of 1 M Tris–HCl buffer of pH 7.5, followed by 2 ml of 0.5% Iodonitrotetrazolium (INT) solution (Sigma product No I 8377).The suspension was kept at 40°C for 1 h and then 10 ml of methanol was added. The samples were mixed and left in the dark for 10 min and, after filtration with Whatman's no 40 paper, the absorbance was measured at 490 nm. Water soluble phenols (WSP) were extracted with distilled water. Dry samples (10 g) were mixed with 100 ml of distilled water and shaken at 75 rev min^{-1} for 20 h at room temperature. Solutions were filtered through Whatman's n°1 paper. All samples were extracted in triplicate. Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin-Ciocalteau reagent, following the method of Box (1983). Tannic acid was used as a standard and the concentration of WSP was expressed as tannic acid equivalents (μg TAE g^{-1} d.s.). Ion concentration was determined by ionic chromatography after extraction of the samples with bidistilled water (soil :water 1:10) for 24 h at 25 °C (Wang et al., 2013) to detect ion concentration (mg g^{-1} dry soil) by using a chromatography systems (Dionex ICS-1100). Osmolarity (OP) of the aqueous extracts of the amended and un-amended soils was measured by the osmometer (Osmolab®One 16S/10S). Cation exchange capacity (CEC) was determined by using the barium chloride–triethanolamine method (Mehlich, 1953). Microbial population was extracted following the method of Insam and Goberna (2004): 2 g of soil were mixed with 20 ml 0.90% NaCl and shaken at 4 °C for 1 h at 8537 rpm to separate microorganisms from solid particles. The suspension was settled for 1 h and the supernatant used for further dilutions with sterile 0.90%

NaCl solution. Dilution factors were determined according to microbial biomass carbon data as suggested by Riddech et al. (2002). Soil fungi population was determined by dilution plate technique (Johnson and Curl, 1972) using Malt Extract agar medium containing chloramphenicol (100 mg ml^{-1}), Sigma Aldrich, Co) (Picci and Nannipieri, 2003) at 10^{-4} dilution in deionized water. Soil bacterial population was determined by Waksman's (1952) method using the nutrient agar medium containing cicloeximide (100 mg/ml, Sigma Aldrich, Co) at 10^{-5} dilution. The inoculated Petri-dishes were incubated at 30 ± 1 °C for 24 h and at 25 ± 1 °C for 5 days for bacteria and fungi, respectively. Three replicate plates were used for each sample.

Statistical analysis

Data are presented as means ± standard error. Data were analyzed by one-way analysis of variance (ANOVA). The significance of the difference from the respective controls for each experimental test condition was assayed by using Tukey's for each paired experiment. Two way ANOVA was performed to analyze the effects of concentrations of both fractions, of soil type and their interaction on OM, N, FDA,DHA, MBC, fungi and bacteria colonies. A $p < 0.05$ was regarded as indicating a significant difference. All data collected were statistically analyzed using SYSTAT 10 software (SPSS Inc.). Pearson's correlations for both soils, digestate fractions and all soil parameters, were carried out using SYSTAT 10 software (SPSS Inc.).

Results and Discussion

Soil properties

The two soils used in this experiment were appositely selected with different chemical and biological properties (Table 1). RIZ soil had a neutral pH, a higher content of water-soluble phenols, organic matter and nitrogen in comparison with SANL.

The C/N ratio was also the highest in RIZ with a value of 10.99. Fungi and bacteria colonies were present in major amount in RIZ than SANL (Table 1). FDA and DHA activities were double in RIZ compared to SANL. Cation exchange capacity was significantly the highest in RIZ while, regarding the nutrients, SANL soil contained calcium, magnesium, potassium and sulphate more than double compared to RIZ soil (Table 1).

Digestate

Both solid and liquid fractions were pathogen and heavy metal free (data not shown). The solid and liquid fractions of digestate significantly differed each other for chemical and biological characteristics (Tables 2, 3).

The solid fraction contained more total solid and volatile organic compounds than liquid fraction, while total phenols amount was lower in DS compared to DL. As known, anaerobic and aerobic microhabitats coexist in soils. The products of the anaerobic process, in terms of organic volatile compounds, are mineralized rapidly (Albers et al., 2018) and can serve as a C source for starved soil microorganisms (Owen et al., 2007), contributing to the increase in soil microbial biomass and activity and in the soil labile carbon, all parameters strictly related to soil fertility. pH values of DS and DL (Table 2) were within the range of 6.7 to 9.2, in line with those reported in literature (Xia and Murphy, 2016), and also the organic matter content was comparable to the values (62–77%) generally reported for agricultural digestates (Monlau et al., 2015a,b). Organic matter, nitrogen percentage and C/N ratio were significantly the highest in the solid fraction (Table 2).

Solid fraction had the highest number of bacterial and fungal biomass. The activities of FDA and DHA, enzyme markers of microbial activity and representative of hydrolytic and oxidative activities respectively, were significantly high in the solid fraction with values, respectively, 4 and 9 times higher in comparison with those of DL. These data evidenced a strict relationship between bacteria, fungi and enzyme activities confirming as the enzymatic activity is an essential part of microbial life (Wlodarczyk et al., 2002). Our results showed also that K^+, Ca^{2+}, Mg^{2+}, NO_3^- PO_4^{3-} and SO_4^{2-} were in the largest amount in the solid fraction (Table 3). Conversely, ammonium was more abundant in the liquid fraction (Table 3).

Influence of digestate fractions on soil chemical and biological properties

Ion concentration is an important parameter regarding the use of digestate, to avoid exceeding maximum land application of potassium 100 kg ha^{-1} y^{-1} and phosphate 60 kg/ ha^{-1} y^{-1} (Rollett et al., 2015). Many previous studies (Edmeades, 2003; Johnston et al., 2009; Diacono and Montemurro, 2010; European Commission, 2019; Muscolo et al., 2017, 2019) pointed out the benefit of the addition of different organic source as a means of increasing organic carbon (OC) and nutrients in soils. In our study, the addition of the digestate fractions influenced the two soils differently and these differences can be ascribed to the diverse intrinsic characteristics of the two soils themselves. Six months after the addition of the different percentages of solid and liquid fractions, ad exclusion of pH, the characteristics of the two soils changed, depending on the percentage and on the type of the fraction used. EC increased, in both soils, when the percentage of solid digestate increased, but at different extent (Tables 4, 5). RIZ soil was non-saline up to the addition of 50% of solid digestate and the soil became moderately saline with 75% DS (Table 4). SANL, already with the addition of 25% DS became moderately saline, to turn highly saline with 50 and 75% DS (Table 5). The differences in OM, pH and CEC, between the two soils, can be the reason of the different

influence on EC of each fraction, as already demonstrated by many authors (Bronson et al., 2005; Sudduth et al., 2005; Aimrun et al., 2009; Peralta and Costa, 2013). However, EC-soil property interactions are not easily identified, since the magnitude of the reactions regulating soil EC levels are complex and dynamic. WSP proportionally enhanced when the percentage of digestate increased in both soils, the greatest WSP content was detected in SANL (Table 5). Min et al. (2015) reported that phenolics in soils were mainly degraded by fungi and bacteria and environmental factors such as neutral soil pH, enzyme activities and soil structure, contributed to faster WSP degradation Our data evidenced in RIZ the presence of all the conditions that favour WSP degradation (Table 4). Organic matter was constitutionally different between the two soils. RIZ had medium content of OM that became elevated since from the addition of the lowest digestate percentage. Differently, in SANL soil the OM content was low, and it became medium after the addition of DS. Nitrogen increased in both soils, much more in RIZ, while C/N increased with 25% DS, and decreased in both soils with the addition of the two highest DS concentrations. The decrease in C/N evidenced as the mineralization process prevailed, due to the great increase in MBC, bacteria and fungi with DS treatment.

Considering the biological soil properties, FDA gradually increased in RIZ compared to control at increasing DS percentage, DHA instead increased up to 50% DS and then decreased (Table 4). Differently, in SANL soil, FDA did not show significant variations compared to control, while DHA activity gradually increased when DS percentage increased (Table 5). The differences related to FDA and DHA between the two soils can be ascribed to the different amount of fungi and bacteria, which are the main producers of the two enzymes (Gaspar et al., 2001; Debnath et al., 2015). CEC increased in both soils, mainly in RIZ at the two highest DS percentages. In addition, Ca^{2+}, Mg^{2+} and K^{+} amount increased in both digestate-treated soils reaching similar values with 50 and 75% DS (Fig. 1). However, the

percentage increase of these cations was considerably higher in RIZ since, before treatment, this soil showed the lowest ionic concentration. Na^+ content increased in RIZ and it did not change in SAN soil. Regarding anions, in SANL the biggest increase was observed (Fig. 1). Six months after the addition of the liquid fractions, the chemical soil parameters also changed but at different extent in the two soils (Tables 6, 7). The increase in pH, EC and OP was related to DL concentrations and was more significant in RIZ compared to SANL soil. Conversely, WSP, organic matter amount and total nitrogen as a percentage of the control, increased much more in SANL (Table 7). The C/N ratio decreased in both soils but much more in RIZ (Table 6). MBC, fungi and bacteria increased both in RIZ and SANL increasing the percentage of both liquid fractions of digestate. Bacteria colonies were more abundant in RIZ while the greatest amount of MBC and fungi was found in SANL. The two amended soils showed also different enzymatic activities, in RIZ the greatest DHA activity was detected, while in SANL the highest FDA activity was found, confirming the strict correlation between the amount of fungi and/or bacteria with the type of enzyme activated. Results of Two-way ANOVA (Table 8) showed that was not the concentration of solid digestate but the type of soil to influence OM, N, FDA, MBC and fungi. The number of bacteria was mainly affected by the concentration while the DHA by the interaction of the concentration x soil type. Conversely liquid fraction concentration affected all soil parameters except OM (F-ratio). These data were confirmed by Pearson's correlation coefficient evidencing that solid digestate in RIZ was not correlated with MBC and DHA, which showed a fluctuating trend going up and down, while in SANL DS was not correlated with FDA. Liquid digestate in RIZ, was not correlated with OM which did not change increasing DL concentrations. In SANL a linear correlation between DL and all soil parameters was observed (Table 9).

CEC did not change in presence of liquid digestate in both soils, but cation concentration changed increasing DL percentage (Fig. 1). Unlike solid digestate, the liquid fraction

increased Na^+ amount more in SANL than in RIZ soil (Fig. 1). Calcium, magnesium and potassium did not change significantly in both soils. Sulphate and chloride anions increased up to similar values in both soil, at the highest DL concentrations (Fig. 1). In short, from these data we can confirm that the correlation of the same digestate fraction with soil parameters changed in respect to the type of soil. Our results are in agreement with previous findings of Makadi et al. (2016) showing as digestate treatments had greater impact in the case of sandy soils than in loamy textured soils. These depend on the capacity of a soil, after the addition of organic material, to hold OC reaching of a new equilibrium, which is related to the intrinsic characteristics of soil itself. In this study, solid digestate resulted better than DL as source of stable organic C able to build-up SOC pools over a relatively short time-frame, but it did not produce the same level of improvement in the two soils, highlighting that it is the soil the main responsible for changes in its own fertility and quality.

Considering that both fractions were free of toxic compounds and in light of the results obtained on the two soils, both fractions can be used to amend soils even if they showed different effectiveness, DS better stabilized the soils increasing CEC and OM, DL speeded the mineralization of organic matter, increasing MBC amount and activity.

Conclusions

In short, the level and nature of the advantages given by the use of digestate fractions as fertilizers depend mainly on soil characteristics rather than quantity (carbon loading) and quality (decomposability) of the organic material applied. These results provide also a robust evidence that the benefits of digestate on soils are dependent on the concentration used. However, Even if the effects of digestate on soil ecosystem were different in extent were both positive and we can expect an economic benefit deriving from the reduction of the costs for its disposal and environmental advantages coming from the non-use of mineral fertilizers. The

solid fraction of digestate could be used as fertilizer or as growing medium for crops considering its positive effects on both soils up to a concentration of 75%. The liquid fraction could be used to fertigation because it provides water and nutrients simultaneously, leading, if properly managed, to the conservation of ground water in an increasingly water scarce world.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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Figure Caption

Fig. 1. Effects of solid and liquid digestate fractions on the concentration of ions in RIZ and SANL soils. Values are means ± SE (n=3). Lower-case refer to differences within RIZ soil and upper-case letters refer to differences within SANL soil. Means followed by different letters are significantly different (Tukey's test at $P<0.05$).

Table 1 Chemical and biological properties of RIZ and SANL soils. Electrical conductivity (EC), water soluble phenols (WSP), osmolarity (OP), organic matter (OM), nitrogen (Ntot), carbon/nitrogen ratio (C/N), fluorescein acetate hydrolase activity (FDA), dehydrogenase activity (DHA), microbial biomass carbon (MBC), cation exchange capability (CEC), sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), chloride (Cl) and sulphate (SO_4). Data were expressed as mean ± standard error. Different letters indicate significant differences at $p<0.05$.

ID	**RIZ**	**SANL**
pH (H_2O)	6.75 ± 0.2^b	8.1 ± 0.3^a
pH (KCl)	5.88 ± 0.1^b	7.55 ± 0.2^a
EC (µS/cm)	50 ± 7^b	65 ± 8^a
WSP (µg TAE g^{-1} d. s.)	10 ± 3^a	2.5 ± 1^b
OP (mOsm)	7 ± 2^a	3 ± 1^b
OM (g kg^{-1}DM)	22.8 ± 0.5^a	10.9 ± 0.2^b
Ntot (g kg^{-1}DM)	1.7 ± 0.05^a	0.6 ± 0.01^b
C/N	10.99 ± 3^a	1.87 ± 2^b
FDA (µg fluorescein g^{-1} d. s.)	16.2 ± 1^a	8.3 ± 0.9^b
DHA (µg TTF g^{-1} h^{-1} d. s.)	65.5 ± 3^a	37.3 ± 2^b
MBC (µg g^{-1}soil)	160 ± 15^a	20 ± 3^b
Fungi (CFU g^{-1})	$1.5*10^4\pm5$	Nd
Bacteria (CFU g^{-1})	$7.0*10^5\pm4^a$	$1.0*10^5\pm3^b$
CEC (cmol(+)/kg)	14 ± 1^a	9.3 ± 0.5^b
Na^+	0.06±0.01	0.07±0.01
Ca^{2+}	0.06 ± 0.02^b	0.24 ± 0.03^a
Mg^{2+}	0.06 ± 0.01^a	0.10 ± 0.01^b
K^+	0.019 ± 0.001^b	0.33 ± 0.02^a
Cl^-	0.02 ± 0.001^a	0.017 ± 0.002^b
SO_4^{2-}	0.03±0.002	0.08±0.02

Table 2 Chemical and biological properties of liquid and solid fractions of digestate. Water content (WC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), electrical conductivity (EC), water soluble phenols (WSP), organic matter (OM), nitrogen (Ntot), carbon/nitrogen ratio (C/N), fluorescein acetate hydrolase activity (FDA), dehydrogenase activity (DHA).

ID	**Solid fraction**	**Liquid fraction**
Total solid %	22 ± 5^{a}	8 ± 3^{b}
Volatile organic compounds (Vol%)	76 ± 2^{a}	67 ± 4^{b}
WC (%)	77 ± 4^{b}	88 ± 3^{a}
pH (H_2O)	8.75 ± 0.2^{a}	7.70 ± 0.3^{b}
BOD (mgL^{-1})	-	8400 ± 29
COD (mgL^{-1})	-	43.000 ± 31
EC (µS/cm)	1457 ± 15^{b}	23245 ± 22^{a}
WSP (µg TAE g^{-1}DM)	550 ± 10^{b}	1185 ± 21^{a}
OM (g kg^{-1}DM)	769 ± 5^{a}	632 ± 3^{b}
Ntot (g kg^{-1}DM)	28 ± 0.5^{a}	9.9 ± 0.2^{b}
C/N	10.99 ± 3^{a}	1.87 ± 2^{b}
FDA (µg fluorescein g^{-1} DM)	161.72 ± 15^{a}	49.61 ± 9^{b}
DHA (µg TTF g^{-1} h^{-1} DM)	951.94 ± 11^{a}	109.70 ± 8^{b}
Fungi (CFU g^{-1})	$2*10^{4}\pm5$	Nd
Bacteria (CFU g^{-1})	$57.5*10^{5}\pm4^{a}$	$6.5*10^{5}\pm5^{b}$

Data were the means ± standard errors. Different letters indicate significant differences at $p < 0.05$.

Table 3 Cations and anions (mg g^{-1} dry soil) detected in liquid and solid fractions of digestate. Data were expressed as mean ± standard error. *Different letters indicate significant difference at $p<0.05$.

ID	**Solid fraction**	**Liquid fraction**
K^+	$6.1\pm1^{a*}$	1.7 ± 0.4^{b}
Na^+	2.5 ± 0.6^{b}	3.5 ± 0.4^{a}
NH_4^+	0.15 ± 0.01^{b}	1.5 ± 0.2^{a}
Ca^{2+}	0.2 ± 0.04^{a}	0.06 ± 0.01^{b}
Mg^{2+}	2.8 ± 0.6^{a}	0.03 ± 0.01^{b}
Cl^-	5.1 ± 0.5^{b}	6.1 ± 0.4^{a}
NO_3^-	1.05 ± 0.04^{a}	0.04 ± 0.01^{b}
PO_4^{3-}	0.98 ± 0.1^{a}	0.05 ± 0.02^{b}
SO_4^{2-}	0.40 ± 0.05^{a}	0.13 ± 0.02^{b}

Table 4 Chemical and biological properties of the soil RIZ 6 months after treatment with different percentage of solid digestate, DS, (25%, 50%, 75%). Electrical conductivity (EC), water soluble phenols (WSP), osmolarity (OP), organic matter (OM) nitrogen (Ntot), carbon/nitrogen ratio (C/N), fluorescein acetate hydrolase (FDA), dehydrogenase (DHA) microbial Biomass (MBC), cation exchange capacity (CEC).

ID	**Control**	**DS 25%**	**DS 50%**	**DS 75%**
pH (H_2O)	6.7±0.2	6.3±0.2	6.4±0.2	6.3±0.3
pH (KCl)	5.7±0.1	5.6±0.1	5.7±0.2	5.6±0.1
EC (µS/cm)	$52±7^{d*}$	$189±15^{c}$	$400±12^{b}$	$799±10^{a}$
OP (mOsm)	$7±0.5^{c}$	$7±0.4^{c}$	$9±1^{a}$	$12±2^{a}$
WSP (µg TAE g^{-1} DM)	$10±3^{b}$	$13±1^{b}$	$15±3^{b}$	$45±7^{a}$
OM (g kg^{-1}DM)	$22±3^{b}$	$34±7^{a}$	$36±2^{a}$	$39±5^{a}$
Ntot (g kg^{-1}DM)	$1.4±0.3^{c}$	$1.7±0.5^{c}$	$3.2±0.6^{b}$	$7.8±0.4^{a}$
C/N	$9.1±3^{a}$	$11.6±1^{a}$	$6.5±0.9^{b}$	$2.9±0.5^{c}$
FDA (µg fluoresc g^{-1}DM)	$18±1^{d}$	$23±1^{c}$	$26±1^{b}$	$31±2^{a}$
MBC (µg g^{-1}soil)	$175±5^{c}$	$215±5^{b}$	$320±8^{a}$	$207±4^{b}$
DHA (µg TTF g^{-1} h^{-1} DM)	$70±3^{c}$	$148.5±5^{b}$	$254.5±7^{a}$	$53±3^{d}$
Fungi (CFU g^{-1})	$2*10^{4}±5^{c}$	$1.9*10^{5}±8^{b}$	$2.5*10^{5}±5^{a}$	$1.9*10^{5}±6^{b}$
Bacteria (CFU g^{-1})	$8*10^{5}±4^{b}$	$8.3*10^{5}±4^{a}$	$8.8*10^{5}±4^{a}$	$8.6*10^{5}±4^{a}$
CEC (cmol(+)/kg)	$14±1^{b}$	$14±2^{b}$	$16±2^{b}$	$23±3^{a}$

Data were expressed as mean ± standard error. *Different letters indicate significant differences at $p<0.05$.

Table 5 Chemical and biological properties of the soil SANL 6 months after treatment with different percentage of solid digestate, DS, (25%, 50%, 75%). Electrical conductivity (EC), water soluble phenols (WSP), osmolarity (OP), organic matter (OM) nitrogen (Ntot), carbon/nitrogen ratio (C/N), Fluorescein acetate hydrolase (FDA), dehydrogenase (DHA) microbial Biomass (MBC), cation exchange capacity (CEC).

ID	**Control**	**DS 25%**	**DS 50%**	**DS 75%**
pH (H_2O)	8.1±0.3	7.5±0.3	7.6±0.2	7.6±0.4
pH (KCl)	7.5±0.1	7.4±0.2	7.5±0.2	7.4±0.2
EC (µS/cm)	$67±7^{d}$	$700±11^{c}$	$838±12^{b}$	$950±15^{a}$
OP (mOsm)	$3±0.3^{d}$	$4±0.2^{c}$	$7±0.8^{b}$	$9±0.7^{a}$
WSP (µg TAE g^{-1} DM)	$2.5±1^{d}$	$15±2^{c}$	$20±3^{b}$	$65±6^{a}$
OM (g kg^{-1}DM)	$11±2^{b}$	$19±3^{a}$	$24±3^{a}$	$24±4^{a}$
Ntot (g kg^{-1}DM)	$0.6±0.1^{b}$	$0.5±0.1^{b}$	$0.9±0.1^{a}$	$1.2±0.3^{a}$
C/N	$10.7±2^{c}$	$27.6±3^{a}$	$15.5±2^{b}$	$11.6±1^{c}$
FDA (µg fluoresc g^{-1}DM)	$8.3±1^{b}$	$9±0.2^{b}$	$10±0.2^{a}$	$9±0.2^{b}$
MBC (µg g^{-1}soil)	$19.8±5^{d}$	$31±9^{c}$	$123±11^{b}$	$175±10^{a}$
DHA (µg TTF g^{-1} h^{-1} DM)	$37±3^{d}$	$95± 5^{c}$	$135±7^{b}$	$206±6^{a}$
Fungi (CFU g^{-1})	nd	$4.3×10^{4}± 8$	$4.2×10^{4}±5$	$4.3×10^{4}± 6$
Bacteria (CFU g^{-1})	$1×10^{5}±4^{b}$	$7.8×10^{5}±4^{a}$	$8.6×10^{5}±4^{a}$	$8.8×10^{5}±4^{a}$
CEC (cmol(+)/kg)	$9.3±1.0^{c}$	$9.4±0.8^{c}$	$13±1^{b}$	$17±2^{a}$

Data were expressed as mean ± standard error. Different letters indicate significant differences at $p<0.05$.

Table 6 Chemical and biological properties of the soil RIZ 6 months after treatment with different percentage of liquid digestate, DL**,** (10%, 25%, 50%). Electrical conductivity (EC), water soluble phenols (WSP), osmolarity (OP), organic matter (OM) nitrogen (Ntot), carbon/nitrogen ratio (C/N), fluorescein acetate hydrolase (FDA), dehydrogenase (DHA), microbial Biomass (MBC), cation exchange capacity (CEC).

ID	**Control**	**DL 10%**	**DL 25%**	**DL 50%**
pH (H_2O)	6.7 ± 0.2^{b}	8.3 ± 0.3^{a}	8.1 ± 0.1^{a}	8.3 ± 0.2^{a}
pH (KCl)	5.7 ± 0.1^{b}	7.6 ± 0.2^{a}	7.7 ± 0.2^{a}	7.7 ± 0.2^{a}
EC (µS/cm)	52 ± 7^{d}	265 ± 21^{c}	586 ± 21^{b}	1130 ± 32^{a}
OP (mOsm)	7 ± 0.5^{c}	16 ± 3^{b}	23 ± 3^{a}	28 ± 3^{a}
WSP (µg TAE g^{-1} DM)	10 ± 3^{d}	20 ± 7^{c}	45 ± 5^{b}	138 ± 7^{a}
OM (g kg^{-1}DM)	22±5	27±3	26±5	27±6
Ntot (g kg^{-1}DM)	1.4 ± 0.3^{d}	2.4 ± 0.5^{c}	4.1 ± 0.4^{b}	6.5 ± 0.5^{a}
C/N	9.1 ± 3^{a}	6.5 ± 2^{a}	3.7 ± 1^{b}	2.4 ± 1^{b}
FDA (µg fluoresc g^{-1} DM)	18 ± 1^{d}	57 ± 4^{c}	86 ± 8^{b}	112 ± 10^{a}
DHA (µg TTF g^{-1} h^{-1} DM)	70 ± 3^{c}	545 ± 15^{b}	569 ± 11^{b}	824 ± 19^{a}
MBC (µg g^{-1}soil)	175 ± 5^{d}	650 ± 6^{c}	1147 ± 10^{b}	1287 ± 17^{a}
Fungi (CFU g^{-1})	$2\times10^{4}\pm5^{d}$	$4.5\times10^{4}\pm5^{c}$	$10.8\times10^{5}\pm7^{b}$	$11.6\times10^{5}\pm5^{a}$
Bacteria (CFU g^{-1})	$8\times10^{5}\pm4^{b}$	$18.5\times10^{5}\pm3^{a}$	$3.8\times10^{6}\pm1^{a}$	$2.6\times10^{6}\pm2^{a}$
CEC (cmol(+)/kg)	14±1	13±2	14±3	13±2

Data were expressed as mean ± standard error. Different letters indicate significant differences at $p<0.05$.

Table 7 Chemical and biological properties of the soil SANL 6 months after treatment with different percentage of liquid digestate, DL**,** (10%, 25%, 50%). Electrical conductivity (EC), water soluble phenols (WSP), osmolarity (OP), organic matter (OM) nitrogen (Ntot), carbon/nitrogen ratio (C/N), fluorescein acetate hydrolase (FDA), dehydrogenase (DHA), microbial Biomass (MBC), cation exchange capacity (CEC).

ID	**Control**	**DL 10%**	**DL 25%**	**DL 50%**
pH (H_2O)	8.1±0.3	7.9±0.2	8.1±0.1	8.2±0.2
pH (KCl)	7.5±0.1	7.3±0.2	7.7±0.2	7.9±0.4
EC (µ±0.03^{b} S/cm)	67±7^{d}	269±5^{c}	381±5^{b}	862±10^{a}
OP (mOsm)	3±0.3^{d}	5±1^{c}	8±2^{b}	14±1^{a}
WSP (µg TAE g^{-1}DM)	2.5±1^{d}	27±1^{c}	57±4^{b}	150±8^{a}
OM (g kg^{-1}DM)	11±2^{b}	15±1^{a}	17±3^{a}	22±0.3^{a}
Ntot (g kg^{-1}DM)	0.6±0.1^{d}	0.9±0.2^{c}	1.8±0.5^{b}	5.0±0.7^{a}
C/N	10.7±3^{a}	9.7±2^{a}	5.49±1^{b}	2.56±0.8^{c}
FDA (µg fluoresc g^{-1} DM)	8.3±1^{c}	97±6^{b}	104±8^{b}	136±11^{a}
DHA (µg TTF g^{-1} h^{-1} DM)	37±3^{d}	253±9^{c}	459±16^{b}	654±13^{a}
MBC (µg g^{-1}soil)	19.8±5^{d}	126±9^{c}	1202±13^{b}	1423±17^{a}
Fungi (CFU g^{-1})	nd	4.3×10^{5}±3^{b}	12×10^{5}±3^{a}	13.8×10^{5}±3^{a}
Bacteria (CFU g^{-1})	1×10^{5}±4^{c}	8.5×10^{5}±3^{b}	7.9×10^{5}±4^{b}	3.5×10^{6}±2^{a}
CEC (cmol(+)/kg)	9.3±1.0	9.1±0.5	9.34±0.3	9.33±0.6

Data were expressed as mean ± standard error. Different letters indicate significant differences at $p<0.05$.

Table 8 Two-way ANOVA results for the effects of different fraction concentrations, of soil type (RIZ and SANL) and their interaction on organic matter (OM), nitrogen (N), fluorescein diacetate hydrolase (FDA), dehydrogenase (DHA), microbial biomass (MBC) fungi and bacteria.

SOLID DIGESTATE							
	OM	Ntot	FDA	DHA	MBC	Fungi	Bacteria
R^2	0.858	0.984	0.991	0.997	0.996	1.000	0.995
F-ratios							
Conc.	13.93***	128.0***	50.17***	758.9***	350.7***	182944***	960.33***
Soil	53.67***	359.6***	1403.9***	39.9***	2119.2***	870187***	n.s.
Conc × Soil	n.s.	83.8***	38.7***	776.1***	149.4***	79878***	3.86*
LIQUID DIGESTATE							
	OM	Ntot	FDA	DHA	MBC	Fungi	Bacteria
R^2	0.730	0.968	0.991	0.999	1.000	1.000	1.000
F-ratios							
Conc.	3.96*	134.18***	262.1***	3436.6***	17405***	555587***	25359***
Soil	28.27***	69.73***	38.9***	980.8***	705***	43024***	9235***
Conc × Soil	n.s.	n.s.	12.8***	123.5***	1028***	10060***	10949***

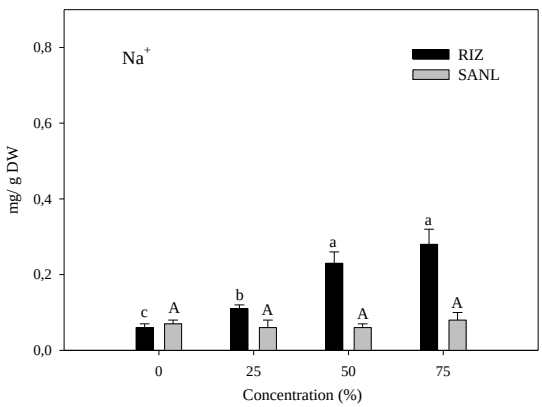
Significance *p<0.05; **p<0.01; ***p<0.001

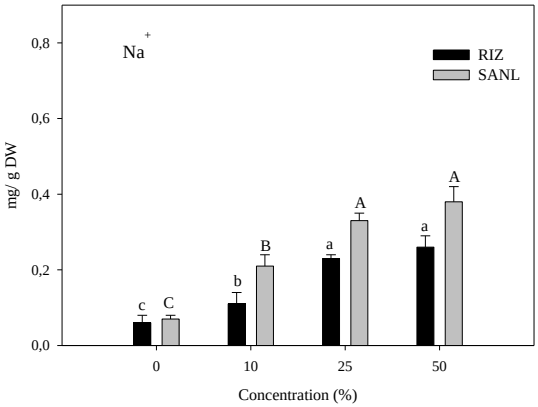
Table 9 Pearson's correlation coefficient (r) between the chemical and biological parameters of RIZ and SANL soils and solid and liquid fractions of digestate.

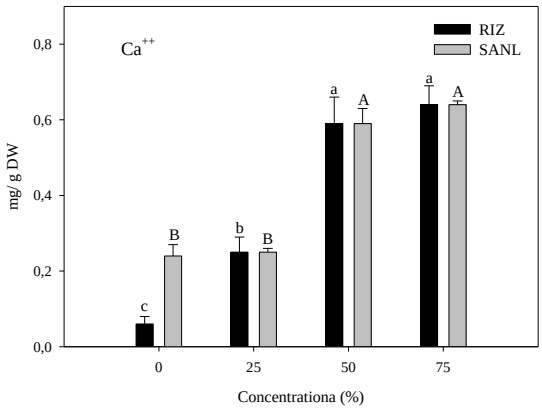
	Correlation Coefficient						
Solid digestate	OM	Ntot	FDA	MBC	DHA	Fungi	Bacteria
RIZ	0.772*	0.894*	0.970**	0.411	0.077	0.742*	0.781*
SANL	0.806*	0.765*	0.449	0.959**	0.992**	0.774*	0.840*
Liquid digestate							
RIZ	0.267	0.982**	0.946**	0.887*	0.916**	0.889*	0.654
SANL	0.883*	0.950**	0.843*	0.974**	0.924**	0.929**	0.940**

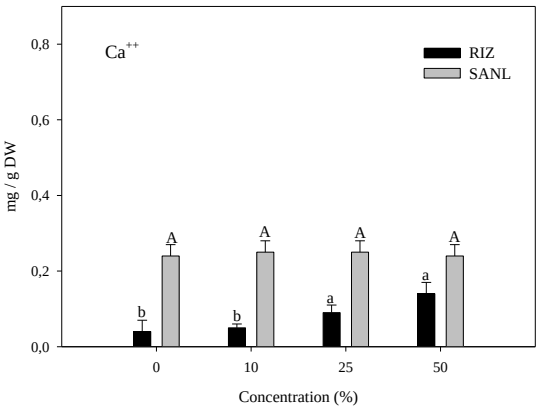
Significant at *P < 0.05; **P < 0.01

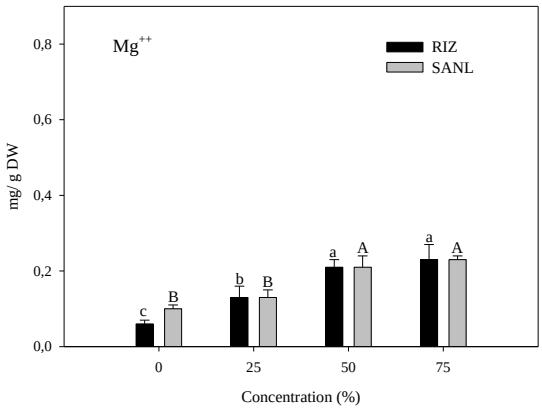
SOLID **LIQUID**

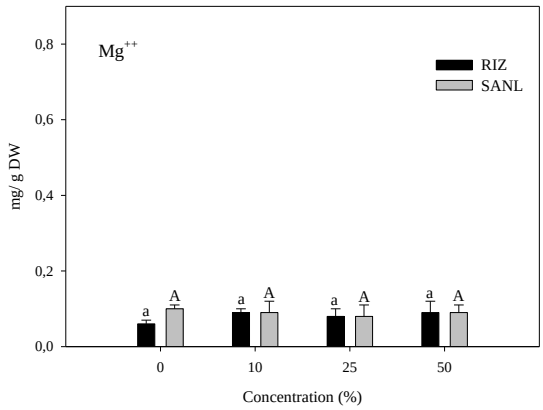












SOLID **LIQUID**