The impact of biogas digestate typology on nutrient recovery for plant growth: Accessibility indicators for first fertilization prediction

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Abstract

In recent years, anaerobic digestion of organic waste (OW) is rapidly appearing as a winning waste management strategy by producing energy and anaerobic digestates that can be used as fertilizers in agricultural soils. In this context, the management of the OW treatment process to maximize agro-system sustainability satisfying the crop nutrient demands represents the main goal. To investigate these traits, two protocols to assess the plant availability of digestate nitrogen (N) and phosphorus (P) were evaluated. With this aim, the N and P availability was determined on 8 digestates and 2 types of digestate-based compost from different OW via sequential chemical extractions (SCE). In addition, the digestates were tested in soil incubations and in plant pot tests with Italian ryegrass and compared with chemical fertilizer and a non-amended control soil. The N extracted from digestates via SCE was related to soil N mineralization and plant N recovery. The C: N ratio had negative impact on mineralized N and its recovery in shoots ($\text{Shoots}_{\text{N}} = -0.0085 \times \frac{\text{C}}{\text{N}} + 0.1782$, r^2=0.67), whereas water extractable mineral N was positevely related to the root N apparent recovery fraction (N-ARF) with ($Roots_N = 5E^{-5} \times N_{solublemin} + 0.0138$, r^2=0.53). The shoot P-ARF was positively correlated with the inorganic water extractable fraction of P ($Shoots_P = 0.1153 \times H_2O - P_i - 0.2777 \times H_2O - P_o + 0.0249$, r^2=0.71) whereas the root P-ARF was positively correlated with the less accessible fractions

($Roots_P = 0.0955 \times NaHCO_3P_o + 0.0955 \times NaOH\ P_o - 0.0584 \times NaHCO_3P_i + 0.0128$, $r^2=0.8641$).

Feedstock digestate typology impacted the N and P recovery results leading to a better description of the typology properties and a first nutrients ARF prediction.

Key words digestates, typology, fertilizers, characterization indicators, nutrient recovery

1. Introduction

An increasing interest exists in improving the soil quality following the utilization of organic wastes (OW) within the environmentally friendly bio-refinery approach (Alburquerque et al., 2012; Gissén et al., 2014). In this respect, anaerobic digestion is a major building block in the circular bioeconomy. Furthermore, both energy (ie. biomethane) and organic fertilizers (ie. digestate) replacing the chemical fertilizers provided to crops can be recovered from anaerobic digestion of OW. From an agronomic and economic point of view, digestate use as a fertilizer can be considered not only as a supplement for traditional organic fertilizers (i.e. slurry) but also as an alternative fertilizer, which under certain soil conditions, is more effective than using NPK fertilizer (Barlog et al., 2019). Indeed, anaerobic digestion is reported as a suitable treatment to easily recover P and N fertilizers from digestate (Mazzini et al., 2020). This is due to organic matter and free organic nutrients biodegraded into mineral forms. However, agricultural reuse of treated OW as soil fertilizers is limited by environmental constraints related to the quality of organic matter, nutrient availability and safety issues. Furthermore, the chemical composition of digestate depends on the nature of feedstock and digestion process (Alburquerque et al., 2012, Guilayn et al., 2019, Barlog et al., 2019). Indeed, a statistical classification of 91 digestates based on their agronomic quality (i.e. C, N, P, total solids, organic matter) was applied on a large range of raw digestates by (Guilayn et al., 2019) and revealed 6 groups of different digestate typology. By analyzing the obtained digestates groups, two criteria impacted the classification: the TS concentration of the digestates associated with dry or wet anaerobic digestion process and the feedstock type, as following: liquid fibrous feedstock (crop residues silage, cattle slurry), liquid sewage sludge, liquid pig slurry, slurries co-digested with silage and green wastes (dry anaerobic digestion), municipal solid wastes (dry anaerobic digestion) and fibrous feedstock (dry anaerobic digestion of cattle manure and green waste). In this framework, mineral N can be readily available for crops but can be temporarily immobilized by microbial biomass during OW mineralization in soil (Lashermes et al., 2010). Therefore, it is important to know the OW mineralization N kinetics to optimize the N supply synchronisation with plant requirement in agricultural systems.

In this context, to better control and manage the quality of digestates, a better knowledge of the accessibility and availability of nutrients and organic matter would improve the fertilizing potential of these products. According to Möller et al. (2012), an accurate characterization of digestate nutrient content and organic matter (OM) composition, combined with experiments to assess the N mineralization and N immobilization processes after field spreading, would be essential for a better characterization of the driving factors driving N turnover in the soil.

Total content of nutrients in OW harldy predicts their fertilizer potential. Ahmad et al. (2018) reported more than 80% of applied P is rapidly immobilized being unavailable for plant following adsorption/precipitation processes or conversion into organic form. To gain a better insight on this issue, many authors proposed to characterize the available P via P fractionation. He et al. (2010) reported that bioavailability of applied P depends on the presence of specific P forms and that labile P includes the sum of inorganic and organic P from H_2O and $NaHCO_3$ extracted fractions. In this regard, Grigatti et al. (2015; 2017; 2019), performed a dedicated SCE on digestates and composts in order to measure the phosphorous potentially available for plants. The authors found good correlations between the water- and the sodium bicarbonate-extractable P from composts with short- and medium-term plant P uptake.

Lashermes et al. (2010) proposed an OW classification based on their chemical characteristics to predict N availability. However, only 2% of the 273 OW studied came from similar typology of digestates coming from fibrous feedtstock anaerobic digestion with low N availability (i.e. digestates was classified in a group with initial N concentration under 65 g kg^{-1} and C: N ratio around 15). However, according to Guilayn et al. (2019), the N content from digestates varies from 20 g kg^{-1} to 175 g kg^{-1} and C: N ratio varies from 2 to more than 30. It would be interesting to complete the

Lashermes study with different typologies of digestate to classify their fertilizer potential. To do that, the first step would be to evaluate the impact of digestate typology on plant growth. Recently, a methodology proposed for organic matter characterization has been applied to a large range of OW in order to predict both bio-avalability and complexity/biodegradability of the organic matter (Jimenez et al., 2015). This technique has been successfully used for OM biodegradation kinetics modelling (anaerobic digestion, compost, soil) (Jimenez et al., 2017) and for organic N dynamics in anaerobic digestion (Bareha et al., 2018; 2019). The basic idea was to find a consistent characterization method in order to describe the bioprocess kinetics and to model the whole treatment chain in terms of organic carbon fate, e.g. anaerobic digestion, compost and organic matter fate in soil. The methodology used is based on SCE to simulate organic matter bioaccessibility for microorganisms combined with three-dimensional fluorescence spectroscopy to analyze the organic matter complexity. The challenge is now to transfer this technique to the characterization of nitrogen accessibility in order to predict its fate after land-spreading and its availability for plant growth.

Apart from the previously mentioned studies, there is no literature information on the development of indicators for both N and P plant availability from digestates. This is the reason this study aimed to propose indicators based on chemical extractions and characterization for N and P.

In this light, the objectives of this work were to determine the plant-available N and P fractions based on characterization of the accessibility study in order to: (i) assess the impact of typology of OW on the nutrient availability, and (ii) use these indicators to predict plant's nutrients recovery. This study, focussed on a wide range of anaerobic digestates from many types of OW. The results could be used for rapid diagnostic of the (N, P) fertilization potential of a digestate.

2. Materials and methods

2.1. Organic waste

In order to ensure a representative survey, the anaerobic digestates used in this work were selected on the basis of the results reported by Guilayn et al. (2019). The typology developed by (Guilayn et al., 2019) was used to select the digestate samples out of a large panel of digestates. For this study, 6 main groups were selected from a statistical study applied to the agronomic characteristics: sewage sludge, municipal waste, cow manure (dry -anaerobic digestion), pig manure (liquid anaerobic digestion), centralised (co-digestion), and crop residues. Table 1 presents the samples used with the type of OW used as feedstock and the conditions of the anaerobic digestion process. Post-treated digestates through phase separation (solid or liquid) and composting were also added. Indeed, phase separation is the most classical digestate post-treatment (Alburquerque et al., 2012), leading to two products of different quality (i.e. solid phase as soil amendment and liquid phase as fertilizer). Besides, two digestate-based types of compost (FFMSW_2 and Sludge_2) were used since composting is widely adopted treatment for the solid phase of urban OW digestate to meet the European fertilizing regulation parameters (European Parliament and Council of the European Union, 2016). The organic waste samples were freeze-dried and ground (ø 1mm), in order to reduce the particle size effect in the soil incubations and plant growth experiments.

2.2. Analytical measurements

Freeze-dried ground samples were further ball milled and analysed for the main physico-chemical parameters. The moisture was determined at 105 ± 2°C until constant weight (24-48h), the volatile solid (VS) was determined on the total solids (TS) at 550 °C for 4 h. The total carbon (TC) and total nitrogen (TN) were determined via an elemental analyser (FlashSmart, Thermo Fisher Scientific). The total nutrient and trace elements were determined by ICP (Inductively Coupled Plasma-OES, Spectro Arcos, Ametek) on ≈250 mg of samples after microwave assisted digestion with 65% HNO_3 + 37% HCl. All the analyses were done in duplicates.

Based on Jimenez et al. (2015), the sequential chemical extractions of organic matter were applied on the freeze-dried and grounded samples (0.5 g). An orbital shaker was used for the extractions steps as following described:30 mL of 0.01M $CaCl_2$ (twice, 1h, 30°C, 300 rpm) ; 0.01M (NaOH+NaCl) (4 times, 15 min, 30 °C, 300 rpm); 0.1M HCl (once, 1h, 30°C, 300 rpm), 0.1M NaOH (4 times, 1h, 30 °C, 300 rpm) and 72% H_2SO_4 (twice, 3h, 30°C, 300 rpm). The fractions names are respectively: Soluble extracted from Particulate Organic Matter (SPOM), Readily Extractible Organic Matter (REOM), Slowly Extractible Organic Matter (SEOM) and Poorly Extractible Organic Matter

(PEOM). The not extracted fraction is called Non Extractible Organic Matter (NEOM). Each extraction was done in two replicates, centrifuged and the supernatant collected and filtered. The recovered pellets were submitted to the subsequent extraction. Ammonium and total nitrogen were measured in the supernatants using HachLange® kits based on colorimetry methods (LCK 303, 2-47 mg N L^{-1}, and LCK 238, 5-40 mg L^{-1} respectively).

The organic products were submitted to P fractionation via SCE according to the method of Dou et al. (2000). Freeze-dried and ball-milled products were extracted for 24 h with deionized water (H_2O) in an end-over-end shaker, and then centrifuged. The supernatants were passed through a Whatman #42 filter, and the recovered pellets were extracted with 0.5 M $NaHCO_3$ (pH 8.5) for 24 h. The same procedure was repeated with 0.1N NaOH and 1N HCl. The residual pellets were treated with a mixture of 96% H_2SO_4 and 30% H_2O_2 via hot acid digestion at 360 °C. Inorganic P (P_i) in the extracts was determined via the molybdenum blue method of Murphy and Riley (1962). The P recovered in each fraction [water extractable P (H_2O-P), bicarbonate extractable P ($NaHCO_3$-P), alkali extractable P (NaOH-P), acid extractable P (HCl-P), Residual-P] was calculated as follows:

$$P_{i\ fraction\ x}\ (\%) = \frac{Pi_{fraction\ x}}{P_{tot\ OW}} \times 100$$ **Equation 1**

where $P_{i;fraction\ x}$ is the inorganic P determined in each fraction (H_2O, $NaHCO_3$, NaOH, HCl, Residual), and $P_{tot\ OW}$ is the total-P determined in the different organic waste via ICP after microwave-assisted acid digestion.

The total recovery was calculated as the sum of all fractions (H_2O-P + $NaHCO_3$-P + NaOH-P + HCl-P + Residual-P) by using the Equation2:

$$Tot\ P_{recovery}\,(\%) = \frac{\sum_{H2O}^{Residual} P_{i\ fraction\ x}}{P_{tot\ OW}} \times 100$$ **Equation 2**

where $\sum_{H2O}^{Residual} P_{i\ fraction\ x}$ represents the sum of the single P recovery values (P_i) in each fraction (H_2O-P +

$NaHCO_3$-P + NaOH-P + HCl-P + Residual-P), and $P_{tot\ OW}$ is the total P determined in the different products via ICP after microwave-assisted acid digestion. Organic P was calculated as the difference between P_{tot} and P_i in the first four fractions.

According to He et al. (2010), H_2O-P, NaHCO3-P, NaOH-P and HCl-P are respectively water-soluble, bioavailable, potential bioavailable (Fe/Al bound) and Ca-bound P.

2.3. X-ray diffraction

Samples submitted to X-ray diffraction analysis were carefully ground and placed in a flat stage sample holder. Data were collected by means of a PANalytical X'Pert PRO powder diffractometer equipped with a fast X'Celerator detector. Cu Kα radiation was used (40 mA, 40 kV). The 2θ range was from 5° to 60° with a step size of 0.1° and time/step of 100 s. Data were processed for phase identification using a HighScore Plus software package (PANalytical).

2.4. Soil incubation

2.4.1. Soil

The soil used for the incubation and pot trial was collected from the top layer in a field in the Po Valley (Bologna, Italy). This soil showed the following characteristics: pH (H_2O 1:2.5), 7.90; particle-size distribution; 184 mg kg^{-1}, sand, 425 mg kg^{-1}, silt, 391 mg kg^{-1} clay; total $CaCO_3$, 85 g kg^{-1}; total organic carbon (TOC), 10.2 g kg^{-1} ; total Kjeldahl nitrogen (TKN), 1.60 g kg^{-1}; C:N, 8.3; exchangeable K, 330 mg kg^{-1} as K_2O, CEC 27.2 meq. 100 g^{-1}. The total (extractable in aqua regia + HF) Al, Fe and P were 35661, 22224 and 808 mg kg^{-1} respectively. The NH_4-oxalate (pH 3) extractable Al and Fe were 764 and 2158 mg kg^{-1} respectively, while the Na dithionite-citrate extractable Al and Fe were 281 and 2462 mg kg^{-1} respectively.

2.4.2. Incubation tests

Soil incubation was conducted in 500 ml plastic vessels with perforated plastic cap, on 250 g of soil (TS basis). The soil was rewetted at 60 % of the water holding capacity (WHC) and pre-incubated for 4 weeks at 25 °C in the dark. Water content was kept at 60% of WHC by weighing the vessels every 2-3 days and by adjusting with water drops if needed. After this period, the organic products (i.e. digestates) were added to the soil at 170 kg N ha^{-1}, defined as the maximum N load per year authorized by the European Nitrates Directive (1991) and accurately mixed by hand. Chemical references used as positive control (Ctrl$^+$) were added by distributing N (as NH_4NO_3) at the same concentration of 170 kg N ha^{-1}, and in addition P and K were added at 117 and 147 kg ha^{-1} (as KH_2PO_4). On the basis of N loading the P added to the soil with the different organic product was on average 20.45 +/- 2.5 mg P kg soil^{-1}. Calculation of the amount of digestate mass added is described by the Equations 3 to 5 considering 0.3 m of soil depth (d) and a bulk density of 1.33 g/cm^3 for 1 ha of soil.

$$\text{dosis}\left(\frac{\text{mgN}}{\text{kg soil}}\right) = \frac{dosis\left(\frac{mgN}{ha}\right)}{area_{1\,ha} \times d} = \frac{170 \times 10^6}{10^4 \times 0.3 \times 1.3 \times 10^3} = 44\ mgN.kg^{-1}$$ Equation 3

$$\text{dosis}\left(\frac{\text{gN}}{\text{kg soil}}\right) = \frac{m_{digestate} \times N_{digestate} \times TS_{digestate}}{m_{soil}}$$ Equation 4

From the Equation 4, digestate amount is calculated as:

$$m_{digestate} = \frac{dosis \times m_{soil}}{N_{digestate} \times TS_{digestate}}$$ Equation 5

$m_{digestate}$ is the digestate mass (kg)

$N_{digestate}$ is the N concentration of the digestate (gN.kgTS-1)

$TS_{digestate}$ is the Total solids content of the digestate (% of fresh matter)

m_{soil} is the considered soil mass in the test (kg)

Three replicates for each treatment were assessed in a completely randomized block design, in addition to an unfertilized treatment control (Ctrl$^-$). Two parallel soil sampling series were done to follow the P and N evolution during incubation. Olsen-P, was determined according to Watanabe and Olsen (1965) at days: 0, 14, 28, 56, 84.The Olsen-P data were used to calculate the relative percentage extractable (RPE) P, used to normalise the P extractability for each treatment relative to Olsen-P obtained for $Ctrl^+$ (Grigatti et al., 2019). The mineral N course in soil was assessed by extracting soil samples (1g TS basis) at days 0, 14, 28, 56 and 84 with 1M KCl for 30 min on an end-over-and end shaker. The solution was filtered over a Whatman #42 filter and ammonium and nitrates were determined using Hach Lange® kits (LCK 304, 0.015-2 mg $N.L^{-1}$ and LCK 339, 0.23-13.5 mg N L^{-1} respectively).

2.5. Plant pot trials

Based on Grigatti et al. (2014 ; 2015), the digestate samples were added to the ground soil at 170 mg N kg^{-1} and thoroughly mixed by hand in 2 liter plastic pots (ø 140 mm × h 150 mm). These were filled with 1 liter of inert material (agricultural light expanded clay), and 1 kg of each different treated soil in 3 replicates. Pots were seeded with 0.8 g of seeds of Italian ryegrass (*Lolium multiflorum* subsp. Italicum), cv. Sprint, covered with a thin layer of sand to prevent drying, watered and placed in a growth chamber at 16-18 h day-night photoperiod at 13–23 °C (±3 °C) day-night temperature, the light was ensured by 6 Philips Master Tld 58 W-840 tubes. Besides the organic products, an unfertilized control ($Ctrl^-$), and a chemical reference (at the same rate used for soil incubation) was added ($Ctrl^+$). The same treatments and dosis performed in soil incubation test were applied. The use of fast growing species as ryegrass in controlled conditions (moisture; temperature; light) leads to a multiple harvest approach giving the opportunity to the best description of apparent N and P utilization kinetics in the time frame of a growing season (Gunnarson et al., 2010; Schiemenz et al., 2010 ; Tampio et al., 2016). After emergence, plants were regularly watered with tap water to keep soil at 60 % Water Holding Capacity (WHC). At each harvest, ryegrass plants were cut 2 cm above ground and collected 3 times: at 28, 56 and 84 days after sowing. The plant biomass was then dried in a forced air oven at 60 °C for 3 days and weighed, to determine dry weight (DW) per pot. Dry biomass was also ball-milled for subsequent analysis. At the last harvest, the roots were divided from the soil with a combined water-sieving separation, and the root biomass was then dried as above, weighed and milled. On plant tissue (shoots and roots), the total P content was determined by means of ICP after (HNO_3, H_2O_2,) microwave assisted digestion. The total N content was determined by elementar analysis as the OW samples. Apparent plant N and P utilization efficiency was calculated on the basis of the Apparent Recovery Fraction (ARF) approach (Gunnarsonn et al., 2010), according to the Equation 6:

$$\mathbf{ARF\_X\ (\%)} = \sum_{i=1}^{3} \frac{\text{X uptake treatment}_{ti} - i\text{X uptake ctrl} -_{ti}}{\text{X added}} . \qquad \textbf{Equation 6}$$

in which X uptake treatment (t_n) is the total nitrogen or phosphorus uptake (mg pot^{-1}) of a fertilizer treatment at time t (i = cut 1-3.); X uptake $ctrl^{-}_{ti}$ is the total nitrogen or phosphorus uptake (mg pot^{-1}) of the unfertilized control at time t (i = cut 1-3); X added is the total nitrogen or phosphorus added to the pot (mg pot^{-1}).

Chemical nutrient equivalent coefficient k_{eq} is defined as the equivalent chemical nutrient dosis (i.e. positive control) percentage needed to reach similar crop yield and is calculated according the Equation 7.

$$k_{eq}X = \frac{ARF\_X}{ARF_{Ctrl+}} \qquad \text{Equation 7}$$

2.6. Statistical analysis

All the data from the plant growth experiment were analyzed by means of Kruskal-Wallis non parametric test. Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) were also performed on all the data using FactomineR package from the R software. Partial Least Square Regression (PLSR) was performed for variables prediction using the SIMCA® software. For PCA analysis, data from Grigatti et al. (2019) were included as far as similar experiments were done on P speciation and plant pot tests. The three samples were D1, D2 and BD, which are respectively digestate from thermophilic wastewater sludge digestion, digestate from mesophilic winery sludge treatment and digestate from mesophilic bovine slurry and energy crops treatment. Table 1 presents these samples, the conditions of the anaerobic digestion process and the associated feedstocks.

3. Results and discussion

3.1. Digestates characterization

The main physico-chemical characteristics of the studied digestates are reported in the Table 2. A high variability of the parameters analysed was apparent. Indeed, organic matter ranged between 40 and and 90% (TS basis), the TKN varied between 15 and 45 g kg^{-1} while P ranged between 4 and 20 g kg^{-1}.The most N-rich samples (44-48 g kg^{-1}) were the liquid phase of the centralised digestate (Centr_2), the pig manure digestate (Agri_2) and the sludge digestates (Sludge_1, D1 and D2). The poorest samples were the digestates of FFMSW and its compost (between 15 and 17 g N kg^{-1}). The richest P samples were the sludge digestate (Sludge_1) and its compost (Sludge_2), along with the solid phase of the centralised digestate (Centr_1) and digestate D2 (sludge from winery processing) with a P content ranging between 18 and 20 g kg^{-1}. The FFMSW_1 digestate and its compost FFMSW_2 besides the silage straw digestate (Agri_3) were the poorest P samples (4 g P kg^{-1}). PCA and HCA analyses were performed on the samples characteristics for a whole sight of the digestates profiles thus allowing the comparison with the typology of Guilayn et al. (2019). The results are presented in the Supplementary Material (Figure A.1). The PCA was applied on nine parameters (i.e from TS to K in Table 2). The first two components recovered ≈ 80% variance. The first component (PC1) was mainly related to the TS, negatively related to OM and K.

PC1 clustered dried (i.e. FFMSW_1, composts Sludge_2 and FFMSW_2) and liquid AD digestates (i.e. all the others). The second component (PC2) was formed by P, which was negatively related to the C: N ratio related to fibrous composition. The sludge digestate samples were associated with P concentration variable whereas Agri_3 (wheat straw digestate) was mainly associated to C: N ratio variable. Furthermore, Sludge_1 and 2 were the samples containing the highest metals concentrations (Fe, Al, Pb, Cu, Mn, Mo, and Cd).

These results were in agreement with the raw digestate typology found by Guilayn et al. (2019). Indeed, Agri_1 and Agri_3 characteristics were in agreement with the characteristics associated to the fibrous feedstock digestate group after dried digestion (i.e. cattle manure and crop residues). Agri_2 characteristics were consistent with those of the pig slurry digestate group from the typology. Sludge_1 characteristics were consistent with those of the sludge digestate group and FFMSW_1 characteristics fit with the municipal solid wastes digestate group characteristics. BW_1 characteristics were mainly close to the biowaste and municipal solids waste digestate composition after dry digestion. However, NH_4 concentration and NH_4: TN ratio had similar values than the manure co-digestion (i.e. NH_4: TN ratio > 70%). This is probably due to the high ratio of agro-industrial waste in the feedstock used in addition of biowastes. Only digestate from the wet digestion of fibrous substrate was not taken into account (silage) in this study. Composts Sludge_2 and

FFMSW_2 and Centr_1 and 2 obtained after phase separation were not compared with the typology as they were not raw digestates.

Attention should be paid to the results for TKN and N-NH^+_4 concentration obtained by the raw sample analysis and to the freeze-dried sample extraction analysis. Regarding some digestates, highly significant positive differences were obtained between raw and freeze-dried samples analyses (from 40% for Centr_2 to 61% for Agri_2 of TKN loss). This result was due to ammonia volatilization during freeze-drying operation. The loss of ammonia could have an impact on soil incubation and plant N recovery results. Considering the 8 others digestates, no significant differences appeared and a linear relation was obtained between raw sample analysis and freeze-dried analysis (i.e. TKN_raw = 0.9443.TKN_freeze-dried, $R^2 = 0.8832$). Furthermore, the N typology of Agri_2 became similar as Agri_1 and 3 (fibrous feedstock involving manures). NH_4: TN ratio evolved from 76% to 39%. This ratio decreased also for Centr_2 from 46% to 9% after freeze-drying. Consequently, Agri_2 and Centr_2 results were used in statistical tests by using the freeze-dried analysis. N-ARF recovery will be discussed accordingly. However, concerning liquid digestates with high ammonia content as pig slurry digestates, the best option would be to perform soil incubation and plant pot trials with fresh matter as Rigsby et al. (2013), de la Fuente et al. (2013). A higher mass of soil would be required to maintain the soil WHC with the liquid digestate moisture, according Equation 5.

This first characterization approach showed that the digestates selected were representative of a large range of digestates (i.e. municipal waste, sludge, manure, crop residue, bio-wastes, centralised).

3.1.1. X-ray powder diffraction

The X-ray powder diffraction has been widely use for the study of the crystalline P species in anaerobic digestate and compost (Li et al., 2019; Grigatti et al., 2017; 2019). In this light the XRD profiles from the tested products can give a valuable insight to their P extractability. The XRD patterns from the digestates are presented in Figure 1. The XRD patterns of samples Agri_1, Agri_2 and Agri_3 were similar showing the presence of crystalline inorganic phases, namely quartz (PDF no. 01-087-2096), calcite (PDF no. 01-085-1108) and sylvine (KCl, PDF no. PDF no.41-1476).

However, these three samples differed significantly in their amorphous phase content, which was definitively higher in Agri_3, followed by Agri_1, whereas sample Agri_2 was mostly constituted of crystalline material. Furthermore, Agri_2 presents also struvite peaks (PDF no. 15-0762), that could be detected on account of the higher crystallinity of this sample. Amongst the analyzed samples, Sludge_1 was the most crystalline one, with no significant presence of amorphous material. The Sludge_1 pattern showed the presence of calcite and quartz as main constituting phases, together with a low amount of vivianite ($Fe_3(PO_4)_2(H_2O)_8$, PDF no. 01-075-1186). Li et al. (2018) found also that quartz was one of the major phases for sewage sludge. Sludge_2 was somewhat similar to Sludge_1, but less crystalline so that only calcite and quartz were detected (in this case quartz is more abundant than calcite). FFMSW_1 and FFMSW_2 were very similar both regarding the amount of amorphous phase which is present together with the fine crystalline fraction of the materials. Regarding the qualitative detection of crystalline phases, it was possible to identify quartz (the most abundant), calcite, together with low amounts of kaolinite ($Al_2(Si2O_5)$ $(OH)_4$, PDF no.01-080-0885) and sylvite. BW_1, Centr_1 and Centr_2 patterns all displayed the presence of quartz, calcite and struvite as crystalline components, but significantly differed for the amount of amorphous material that was relatively low in Centr_2, and higher and similar in BW_1 and Centr_1. The relationship between the XRD outocomes and the P extractability are dicussed in the following section.

3.1.2. Sequential chemical extractions of Nitrogen and Phosphorous

The N fractionation protocol used in this work was developed by Jimenez et al. (2015), and has been validated to describe OM and N evolution during biological degradation processes (Jimenez et al., 2017 and Bareha et al., 2018). The P fractionation has been adapted from Hedley et al. (1982), using methodology by Grigatti et al. (2015, 2017, and 2019). These authors showed the link between labile-P from organic waste samples and the plant P-uptake. The N and P speciations have been assessed applying both fractionation protocols to the selected digestates (Figure 2 a and b respectively). The results showed that the digestates performed different N and P speciations. This was related to the digestate typology and the nature of the feedstock for anaerobic digestion, according to Guilayn et al.

(2019). The observed variability in N and P speciation showed the significant effect of the feedstock on the tests discussed in section 3.2. In the study of N accessibility (Figure 2 a), mineral nitrogen was assessed in the water extractable fraction SPOM. The non-extractable organic nitrogen varied between 25 and 48% except for Agri_3 with only 5% N in the NEOM fraction.

HCA was applied on the N speciation data (not shown). Four groups were found, ordered by N accessibility basis as follows: (i) Group 1: High NH_4-N SPOM samples: Centr_1 and BW_1. This result is consistent since these samples had poor TS; (ii) Group 2: High organic SPOM and REOM: Agri_2 and Sludge_1. SPOM and REOM are mainly composed of accessible and readily extractable proteins; (iii) Group 3: High organic SEOM: Agri_3. SEOM is mainly composed of complex proteins and humic-like substances; (iv) Group 4: High PEOM organic N content samples: Agri_1, FFMSW_1, Centr_2, FFMSW_2 and Sludge_2. PEOM extraction targets holocellulose-like compounds found in fibrous digestates as Agri_1, FFMSW_1 and 2. Phase separation concentrates OM and fibers in the solid phase (Guilayn et al., 2019) as Centr_2. Finally, the compost Sludge_2 was also clustered in this group because of its co-composting with green wastes.

P was extracted in each fraction as reported in Figure 2 b. Phosphorus fractionation showed that inorganic P content was higher than organic P in all the fractions, as reported in the studies of Grigatti et al. (2015, 2017 and 2019) on digestates and composts, and as observed by He et al. (2010) on poultry litter and dried wastewater sludge. Mazzini et al. (2020) showed that 78 to 93% of TP was inorganic following the SCE from six types of animal slurries digestates. The authors showed relevant NaOH (5-43% of organic P) and HCl (2-25% of organic P) extractable organic P, relating this to inorganic P microbial immobilization during anaerobic digestion for microorganism growth. Indeed, soluble inorganic P would be transformed into organic P compounds such as phosphates monoesters or DNA. This biological organic-P may be rapidly mineralized once in soil (He et al., 2010). In this context, NaOH and H_2O were the most organic-P rich fractions from the samples investigated in this work. Furthermore, Agri_1, 2 and 3, and FFMSW_1 mainly showed NaOH extractable organic P. Agri_3 contained the highest organic P content (≈40%), and 1.5 to 4- folds higer than other samples. In water Agri_2 and 3 showed 60% of organic P, higher than BD (49%).

The results obtained by He et al. (2010) on poultry litter showed that a large part of organic P was in the NaOH fraction and was mainly related to phytate-like. Mazzini et al. (2020) showed similar results on crop residues digestates. The authors showed also that organic P were mainly extracted in NaOH fraction of 6 agro-wastes digestates as shown by Agri_1 and 3. Organic P was observed in HCl fraction from Sludge_1 and 2 in this study. He et al. (2010), observed also organic P in HCl fractions related to non-hydrolysable fractions in the dried wastewater sludge. The $NaHCO_3$ and the HCl fractions showed to be the most inorganic P rich factions from the samples tested in this work thus fitting to available P and Ca-bounded P. The labile-P fraction (H_2O + $NaHCO_3$) was found at the highest level in the agricultural residue digestates (Agri_1, 2 and 3). Similar observations on bovine manure and energy crops digestate (BD) were made by Grigatti et al. (2019). Then the biowaste and agro-food industries digestates (BW_1, Centr_1 and 2) had also high labile-P. The FFMSW samples showed intermediate P accessibility. The sludge samples were characterized by a high NaOH extractable P, related to metal-bounded P. Indeed, this result was consistent with both Al and Fe content ($\approx$7 and $\approx$37 g kg^{-1}). Li et al. (2018), showed mainly NaOH extractable Al, while Fe was NaOH soluble being also extracted by HCl in sewage sludge, thus showing Fe-bounded P was partially occluded. Amongst the others samples, compost Sludge_2 showed important poorly available HCl-P, being very similar to FFMSW_2, these results were consistent with the P fractionation of composts showed by Grigatti et al. (2015, 2017, and 2019).

Finally, HCA showed six groups (clustering not shown). Groups were ordered according to the chemical availability level (i.e. Labile-P> NaOH-P> HCl-P) as follow: (i) Very high P accessibility related to high H_2O-P fractions: digestates from liquid feedstock (no manure), biowastes and winery (BW_1, D1 and D2). These samples were characterized by amorphous P and crystalline P (quartz and struvite); (ii) High P accessibility associated with organic H_2O-P and $NaHCO_3$-P: digestates from liquid manure (BD and Agri_2). These samples were characterized by crystalline P (struvite); (iii) Moderate P accessibility (intermediary P speciation): digestates from organic fraction from municipal wastes digestates and centralised digestates (FFMSW_1, FFMSW_2, Centr_1 and Centr_2). These samples were characterized by an amorphous phase and mainly quartz and calcite as crystalline phases; (iv) Poor-P accessibility associated with organic $NaHCO_3$-P and NaOH-P, associated with digestates from fibrous substrates as cow manure with crops residues and wheat straw (Agri_1 and Agri_3). These samples were characterized by a high amorphous phase; (v) Very poor P accessibility associated with inorganic NaOH-P and low fraction of available P: digestate from solid phase of sludge (Sludge_1). This sample was characterized by a crystalline P phase (mainly quartz and calcite); (vi) The poorest accessible P associated with inorganic HCl-P was compost of sludge digestate (Sludge_2). This sample was characterized by less crystalline P than Sludge_1 with calcite and quartz.

For some groups, the feedstock type seemed to have an impact on the P accessibility of digestates (solid phase of sludge, liquid manure, liquid feedstocks, fibrous feedstock). Another observation was that the amorphous and crystalline characteristics also seemed to be associated with some groups: fibrous digestates were mainly composed of amorphous phase and contained organic P in $NaHCO_3$ and NaOH. The labile P fractions had in common amorphous P and struvite as crystalline P. The most crystalline P-rich fractions were found in the solid phase of sludge digestates before and after composting, containing low available P and high sparingly soluble HCl-P.

The eight tested digestates represented a wide range of inherent characteristics and accessibility patterns for both N and P. In this framework, the soil and plant test results can give a deeper insight to these issues as discussed in the following section.

3.2. Soil incubation and plant pot trials

Table 4 shows the cumulative amount of biomass harvested in ryegrass plant pot tests. Any significant differences between treatments for tissue and root (Kruskal-Wallis test p=0.89 and 0.23 respectively). Nevertheless, it appears that poor biomass was obtained in Agri_2 treatment (pig manure digestate), and in FFMSW_2 treatment (FFMSW digestate compost), close to the negative control. The best results were obtained in Centr_1 and BW_1 (solid phase of a centralised digestate and biowaste digestate). The total plant biomass (shoot+ root) was the highest (g pot^{-1}) in Centr_1 (4.11) ≥ Sludge_2 (3.75) ≥ BW_1 (3.72) ≥ FFMSW_1 (3.70) ≥ $Ctrl^+$ (3.65) ≥ Sludge_1 (3.48) ≥ Agri_3 (3.38) ≥ Centr_2 (3.23) ≥ Agri_1 (3.18) ≥ Agri_2 (3.02) ≥ FFMSW_2 (2.93) ≥ $Ctrl^-$ (2.90).

3.2.1. Nitrogen fate

The N mineralization data from soil incubation (Figure 3) showed the net cumulated mineral N (i.e. $NH_4 + NO_3$ mass) during soil incubation (Figure 3a) and the net cumulated mineralization rate of organic N (Figure 3b).

Concerning the available mineral N (N-min), BW_1, Centr_2, Sludge_1, Sludge_2, Agri_2 and FFMSW_1 achieved the highest cumulated values after 84 days of incubation. On the contrary, Centr_1, FFMSW_2, Agri_3 and Agri_1 performed poorly cumulated N-min. These latter were more fibrous samples, with high C: N ratios.

The cumulated organic N mineralization rate was calculated (Figure 3b). Two groups of treatments appeared and were classified in the same order than the cumulated N-min: (i) positive mineralization rate associated with the Sludge_1, Centr_2, BW_1, Agri_2, Sludge_2 and FFMSW_1 treatments and (ii) negative mineralization rate associated with Centr_1, FFMSW_2, Agri_1 and Agri_3. Sludge_1 showed the highest N mineralization rate (30%) suggesting that this digestate was rich in hydrolysable proteins. Organic N from the liquid digestates Centr_2 and Agri_2 showed respectively 21% and 18% of mineralized organic N, following Sludge_1 (30%). This result is consistent with Guilayn et al. (2019) who reported that the highest organic N came from sludge digestate and pig-slurry digestate group. Sludge_2 and FFMSW_1 performed lower N mineralization rates (11%). Similar results were observed in liquid or solid digestates by Rigsby et al. (2013). They showed that the solid phase from municipal solid wastes digestate (C: N ratio of 11) had negative N mineralization rate whatever the soil composition used (sandy, silty or clay). On the contrary, Rigsby et al. (2013) showed that the liquid digestates from slurry (C: N ratio of 4), had 16% to 40% N mineralization while food waste digestate (C: N ratio of 14) reached 30% N mineralization.

The negative mineralization rates group was associated to an immobilization of mineral nitrogen by the soil microorganisms during their growth (De la Fuente et al., 2013). This was related to the increased microbial activity following the OW addition to the soil. Indeed, nitrogen is the main limiting nutrient for plant growth, especially for a fast growing, highly demanding species such as Italian ryegrass (Grigatti et al., 2011). These results are in agreement with Cavalli et al. (2017) which reported immobilization for high C: N ratio anaerobic digestates. The authors showed the low C: N ratio (5 to 7) associated to the raw digestate and its liquid phase (cattle slurry/mais silage) induced net

N mineralization (≈ 30%). On the contrary, the cellulose- and volatile fatty acids-rich solid phase (C: N ratio, 20) induced lower net N mineralization (9–16%). Indeed, negative correlations were found between the N mineralization rate and C: N ratio (r= - 0.73, p = 0.029) as observed by Morvan et al. (2006) on animal manure wastes.

Considering the impact of process treatment of digestates, composting appeared to negatively affect N mineralization. N mineralization rate from Sludge_2 (11%) was lower than Sludge_1 (30%) and FFMSW_1 (11%) had highest mineralized values than FFMSW_2 (-2%). Phase separation impacted also organic N mineralization as shown by Cavalli et al. (2017). Solid phase (Centr_1) reached negative values of minerlization whereas Centr_2 reached 21% of mineralized N.

Plant growth experiments were affected by fertilizer treatment for N-ARF (Kruskal-Wallis, p = 0.026) only for shoot plant tissues. Cumulated N recovery by plant tissues was plotted in the Figure 4. The chemical treatment showed the greatest shoot N recovery (70%) with a total of 76% of N-ARF. N uptake recovery was mainly observed in shoots (64% to 87% of the total recovery). Root N-ARF was measured between 7% and 36% of the total N-ARF, except for Agri_1 and Agri_3 treatments where negative to zero values were obtained.

Sludge_1 and Sludge_2 achieved the best N-ARF amongst the digestates treatments (16.5 and 15.1%). These results were consistent with the Sludge_1 treatment yielding the highest N-min available for plant growth. Centr_2 (13.7%), BW_1 (13.2%) and Centr_1 (11.6%) treatments showed intermediate total N recovery. The FFMSW_1, FFMSW_2 treatments followed with 8.2% and 6.4% respectively. Finally, a last group formed by Agri_1 and Agri_3 was observed with low N-ARF of 1.8% and 2.6% respectively. In soil incubation tests, Agri_1 and 3 showed N immobilization. Accordingly to these outcomes, the plant N-ARF was negative or close to 0 and close to the unfertilized soil treatment.

Considering the digestate treatments impact, composting (i.e. FFMSW_2 and Sludge_ 2) lowered the fertilizing N value of its associated digestate (i.e. FFMSW_1 and Sludge_1 respectively). Phase separation impacted also the N fertilizing potential as shown by Tambone et al. (2017). Centr_2 treatment induced higher N mineralization rates and N-ARF than Centr_1 treatment. According to N speciation and characterization, the solid phase Centr_1 contained more fibers than the liquid phase Centr_2.

PCA and HCA were performed to find correlations between the N availability data, digestate characteristics, mineralized N percentages and N-ARF from shoots and roots as reported in Figure A.2 a (supplementary material). No significant correlation was observed between the total N content, the mineralization rate and the N-ARF. However, a strong correlation was obtained between the C: N ratio and N-ARF from shoots-N (r=-0.82, p=0.004) as in the mineralization N tests without plants. This was described by a linear equation (Equation 8) based on the data of this study and validated by data from digestates and composts studied in Grigatti et al. (2015). Similarly, Decoopman et al. (2017) found a correlation between C: N ratio of agricultural digestates and plant N-ARF measured in field experiments on cereals.

$$\mathrm{Shoots_N} = -0.0085 \times \frac{C}{N} + 0.1782,\ r^2 = 0.6718 \qquad \text{Equation 8}$$

The fiber component of digestates had a negative impact on mineralised N and N recovery by plant tissues. Similarly, the fiber fraction PEOM of digestates was negatively correlated with cumulated mineralized N (-0.58, p=0.08) and shoots-N although to a lesser extent (r=-0.58, p = 0.44). Moreover, cumulative mineralized N and shoots-N ARF were positively correlated (r=0.72, p=0.02) which was consistent with the availability of N for plant growth. Concerning roots, there was a positive correlation between roots N-ARF and nitrates content (r=0.72, p=0.017), SPOM_NH_4 (r= 0.61, p=0.06) whereas SPOM_org and roots_N ARF were negatively correlated (r=-0.59, p=0.07). A linear equation (Equation 9) was found between Roots N-ARF and water extracted mineral N (i.e. $N_{soluble\_min} = NO_3^- + \mathrm{SPOM\_NH_4}$).

$$\mathrm{Roots_N} = 5E^{-5} \times \mathrm{N_{solublemin}} + 0.0138,\ r^2 = 0.5313 \qquad \text{Equation 9}$$

According to Gunnarsson et al. (2010), plants respond to N availability with a different root/shoot nutrient allocations and plant growth rate could be influenced by the ammonia/nitrates ratio in soil solution due to the different N sources. Authors observed an increase in root biomass as a result of the availability of a high amount of ammonia.

HCA revealed four clusters (Supplementary material Figure A.2.a) as follows: (i) Group 1: Agri 3 associated with fibrous digestate with high C: N (>25), high SEOM_N and PEOM_N and low shoots-N ARF. This type of digestate is not suitable as N fertilizer; (ii) Group 2: FFMSW_2 and Agri_1 15<C/N<13, intermediary protein-like and fibrous substrate digestates with high organic SPOM_N and PEOM_N for Agri_1 as compost FFMSW_2. They recovered very low roots N-ARF and intermediate level of shoots N-ARF; (iii) Group 3: Centr_1, FFMSW_1 and BW_1 municipal solids and biowastes digestates, with C: N ratios of 15 in average, high ammonium and nitrate levels in the labile fraction and high levels of total N recovery; (iv) Group 4: The protein-like digestates (Sludge_1, Centr_2, Agri_2) which have a poor C: N (<10), poor PEOM_N, high SPOM_Norg content and the highest total N recovery. Sludge_2 was classified in the Group 4 because of its lower C: N ratio (9.7) and its high N mineralization level.

C: N ratio is associated to the fibrous level of an OW and seemed to be discriminant enough to predict N-ARF and organic N mineralization rate. Digestates with C: N ratios ≥15 were not suitable as N fertilisers whereas digestates with lower C: N ratios had a high potential of N fertilizer.

Accessibility fractionation of N allowed a consistent digestate classification. Furthermore, interesting correlation between roots-ARF N and water extractable nitrates and ammonia were found.

3.2.2.Phosphorous fate

The Olsen-P course during the soil incubation is shown by Figure 5a. The Olsen-P evolution in soil depends on several phenomena: (i) the physico-chemical conditions of the soil can trap some weakly bound P, (ii) the organic part of P can be mineralized and (iii) P can be taken up by soil microorganisms for their growth.

The control soil had the lowest Olsen-P content throughout the incubation (12-14 mg kg soil^{-1}), whereas the compared treatments exhibited different Olsen-P courses in time. All the tested samples showed an available P depletion during the first two weeks. The chemical control (Ctrl^{+}) performed the fastest depletion. Indeed, the chemical P treatment (phosphate salt) can be rapidly fixed with Ca components in calcareous soil (Alburquerque et al., 2012). In this context, the presence of organic matter and the different P forms from digestates can have positive interactions with soil, performing lower fixation and higher efficiency in comparison to chemical P sources (Alburquerque et al., 2012).

In some treatments, an Olsen-P increase appeared between 14 and 28 days (Agri_2, Centr_1, BW_1 and FFMSW_1) probably due to organic P mineralization before another decrease until 56 days. This latter decrease led to a lower quantity of available P for plant growth. The RPE obtained was between 50 and 84%. The RPE of the Centr_2 treatment was the highest with 84% vs. the chemical P treatment, followed by Sludge_2 (74%). FFMSW_1, Agri_2 and Agri_3 were the samples where the P fixation was the highest (RPE of 60% in average). The other digestates were intermediate between the two groups. FFMSW_1, Agri_2 and Agri_3 contained high fractions of water P.

The pot tests showed the treatments affected the plant P tissue (p-value = 0.021). In this context the control soil performed the worst at the first cut (1.93 $mg.pot^{-1}$), showing very poor performance during the plant pot test reaching 4.55 mg pot^{-1} on average (Figure 5b and 5c). The shoot-P ARF is shown in Figure 4a and b. P-ARF from Agri_2 and Agri_3 was negative from the beginning. This result was consistent with the soil incubation observations. This can be linked with high fixation of P in soil as suggested by Ahmad et al. (2018). In the second cut (day 56), the cumulative shoots P-ARF decreased in all the treatments. Only FFMSW_1 and Centr_2 showed opposite outcomes. Then, between the second and the third cut (day 56; 84), shoot-P ARF further increased. In the end the whole P-ARF (%) was: Centr_1 (5.78)≈ FFMSW(5.31)≥BW_1 (4.35)>Centr_2(3.78)>Sludge_1 (2.72)≥Agri_1 (2.44)≥Sludge_2 (2)>FFMSW_2 (1.6)>Agri_2 (0.6)≈Agri_3 (0.5%).

FFMSW_2, Agri_2 and 3 showed lower shoots P-ARF than the chemical treatment (1.81%). That means they were not suitable for P fertilization on calcareous soil.

The root-P content was unaffected by the treatment. However, the root P-ARF (%) was: Agri_1 (2.46)> Agri_3 (2.04)> FFMSW_1 (1.18) = Sludge_1 (1.17) = Sludge_2 (1.16)> Centr_1 (1.04)> BW_1 (0.3)> Centr_2 (0.00)> FFMSW_2 (-0.08)>Agri_2 (-0.21). Shoots-P and roots-P recovery potential were different depending on the treatment, except for Agri_2 and FFMSW_2 which had the lowest ARF P recovery for both.

Briat et al. (2020) reported that availability of soil P (and K) for plants highly depends on the N availability. They reported that P concentrations on shoots were correlated with N concentrations in shoots. Indeed, a significant correlation was observed in this study between total N-ARF and total P-ARF ($r = 0.8152$, $p = 0.0022$) with a linear relationship (ARF_N/ARF_P = 7.7535, $r^2 = 0.6626$) thus proving N and P recovery by plants were linked.

PCA and HCA analysis were performed on both shoots and roots P uptake and P speciation for the 8 digestates studied beside the three digestates from Grigatti et al. (2019), as shown by Figure A.2 b in Supplementary Material. Six groups were found according HCA analysis. The results showed that the P-ARF was classified not only according to the digestate's nature but also according to their P accessibility. The groups found were: (i) group 1 : Sludge_2, compost of solid phase of sludge digestate, HCl rich with an intermediate level of P recovered in shoots and roots; (ii) group 2 : Sludge_1, solid phase of sludge digestate (inorganic $NaHCO_3$ and NaOH fractions rich), with an intermediate level of P recovered in shoots and roots; (iii) groups 3-4 : Centr_1, FFMSW_1, FFMSW_2, Centr_2, Agri_2 (intermediate to low total P-ARF); (iv) groups 5-7 : BW_1, D1, D2 and BD (high total P-ARF and highest inorganic H_2O-P); (v) group 6 : Agri_1, Agri_3, fibrous digestates. Agri_1 has high P total recovery in both shoots and roots as BD, but Agri_3 shows a high recovery only in roots. It seems that the fibrous characteristic enhances P recovery above all in in plants roots through the organic fraction of NaOH-P.

The groups obtained were similar to digestate typology groups, except for Agri_2 and BD which were not in a same group. When doing statistical treatments on the shoots results and P speciation for the 8 digestates used in addition to the 3 digestates used by Grigatti et al. (2019), some correlations were found. Water-P fraction was positively correlated with shoots ($r=0.49$, $p=0.09$). This result was also observed by Grigatti et al. (2017) for composts. As reported by Ahmad et al., 2018, available P consists in the exchangeable and soluble P corresponding to water and Na-bounded P fractions (H_2O+ $NaHCO_3$) whereas occluded P consists into P bound with metals (Fe, Al) and Ca. However, a simple linear model did not fit between inorganic H_2O-P fraction (H_2O-P_i) and shoots P-ARF. PLS model was used showing that two main significant variables were needed to predict shoots P-ARF (Equation 10). H_2O-P_i fraction impacted positively shoots P-ARF, as expected, whereas organic H_2O-P fraction (H_2O-P_o) had a negative impact. Therefore, available form of P for shoots growth was associated to H_2O-P_i fraction, as expected. H_2O-P_o fraction is accessible but has to be mineralized to be available.

$Shoots_P = 0.1153 \times H_2O\ P_i - 0.2777 \times H_2O\ P_o + 0.0249$, r^2=0.71 Equation 10

No correlations were found between ARF results and TP or phosphates concentrations (r=-0.12, p=0.74, r=-0.26, p=0.46 respectively) meaning that the TP concentration is not enough to predict P uptake by plants.

Organic $NaHCO_3$-P fraction ($NaHCO_3$-P_o) was positively correlated with roots (r=0.62, p=0.025 with D1, D2 and BD). Organic NaOH-P fraction (NaOH-P_o) was also positively correlated with roots (r=0.79, p=0.006). A PLS regression model was found between roots P-ARF and inorganic and organic P content in $NaHCO_3$-P and NaOH-P fractions (Equation 11).

$Roots_P = 0.0955 \times NaHCO_3P_o + 0.0955 \times NaOH\ P_o - 0.0584 \times NaHCO_3P_i + 0.0128$, r^2=0.8641

Equation 11

Negative impact of inorganic labile $NaHCO_3$ was found whereas organic P forms impacted positively the roots P-ARF. As previously mentioned, phytate and others phosphates esters can be extracted in the NaOH-P fraction (He et al., 2010). Roots can exude phosphatases able to hydrolyse phytate and organic P forms able to be absorbed by roots (Lambers et al., 2006; Gerke et al., 2015).

Interestingly, roots and shoots P recoveries were correlated with different P fractions. Finally, chemical nutrient equivalents were calculated for all the treatments. The obtained values were above 100% except for Agri_2 (35%) and Agri_1 if only shoots were considered. That means that all the studied digestates (except from pig slurry) can substitute chemical P needs.

Similarly to N results, phase separation impacted the P fertilizing potential. The solid phase of centralised digestate Centr_1 was more suitable as P fertilizer than Centr_2. Composting impacted also the fertilizing value of the municipal waste digestates by lowering the P fertilizer potential relative to its respective digestate. This trend was also showed by Sludge_1 (digestate) versus Sludge_2 (composted digestate). Knowledge of the availability of P and its effects makes possible to anticipate fertilisation according to the soil composition (Alburquerque et al., 2012) and not only based on overall P analysis. Indeed, this was the case of sludge digestates Sludge_1 and 2 which had the highest P contents (same level of Centr_1) but their treatments obtained moderate P-ARF. From P results, first guidelines can be given according to the digestate feedstock typology for the studied calcareous soil and ryegrass. Pig manure digestate (Agri_2) seemed not suitable for P fertilization. Fibrous digestates rich on wheat straw Agri_1 and 3 had very low P fertilizing potential whereas FFMSW_1 presented higher potential as the solid phase Centr_1. The compost FFMSW_2 had also low P fertilizing potential and the liquid phase Centr_2 was intermediate. Sludge 1 and 2 had intermediary results and biowaste digestate BW_1 showed high N and P fertilizing potential.

4. Conclusions

This study focused on different typologies of digestates classified according to their process and to their feedstock. In this context, both P and N speciations showed a wide accessibility range according to feedstocks charateristics. The chemical accessibility indicators described the nutrient availability for plants and allowed the digestates classification on N and P fertilizing potential basis. The N and P speciation impacted the results from incubations with bare soil as well as the apparent coefficients of the use of N and P by the plant for its growth. Depending on the tissue collected (shoot or root), the speciation variables having a significant impact were different for P and N, for the type of calcacerous soil used. First models were found to predict P recovery in shoots and roots using P speciation. Furthermore, C: N ratio value was significant and could be used for shoots N-ARF prediction wheras mineral water extracted N could be used for roots N-ARF prediction. Thus, a more detailed knowledge of the digestates would allow more adequate control of fertilization. Moreover, composting and phase separation impacted the nutrient recovery and can be used as an actuator to propose different organic fertilizers type. In terms of perspectives, field trials on contrasted soils qualities for crops with contrasting nutrient needs should be carried out in order to offer a guide to fertilization by type of digestate. Finally, N and P speciation studied could be used in dynamic models to improve soil and plant model predictions for digestate use in agriculture.

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Table 1: Definition and origins of digestate samples

Sample Name	Type	Scale	Anaerobic Digestion conditions: type (dry or liquid), feed mode (batch or continuous) and temperature	Digester Hydraulic retention time	Post-treatment	Substrate	Reference
Agri_1	digestate	Operating scale (farmer)	Dry batch mesophilic	60 days	-	Cow manure	This study
Agri_2	digestate	Operating scale (farmer)	Liquid continuous mesophilic	60 days	-	Pig manure co-digested with energetic crop and vegetable residues	
Agri_3	digestate	Lab scale	Dry batch mesophilic	100 days	-	wheat straw silage	
Sludge_1	Solid phase from digestate	Operating scale (private agency)	Liquid continuous mesophilic	20 days	Press filter	wastewater treatment sludge	
Sludge_2	compost of solid digestate	Operating scale (private agency)	Liquid continuous mesophilic	20 days	Press filter and composting	digestate of wastewater treatment sludge (1/3) and green wastes (2/3)	
FFMSW_1	digestate	Operating scale (private agency)	Dry continous thermophilic	20 days	-	Fermentable fraction from municipal solid waste (FFMW)	
FFMSW_2	compost of digestate	Operating scale (private agency)	Dry continuous thermophilic 20	20 days	Compostin g	96%FFMSW, 50% green waste, 14% agro-industrial wastes	
BW_1	digestate	Operating scale (governmental)	Liquid continous mesophilic	60 days	-	Biowastes (60%) from supermarkets co-digested with agro-industrial wastes (28%) and crop residues (12%)	
Centr_1	Solid phase of digestate	Operating scale (governmental)	Liquid continous mesophilic	45 days	screw press	Oil (20%), crop residues (10%), agro_industrial wastes (55%), sewage sludge (10%), biowaste (5%)	
Centr_2	Liquid phase of digestate	Operating scale (governmental)	Liquid continous mesophilic	45 days	screw press		
D1	Digestate	Operating scale	Liquid continous thermophilic	na	-	Wastewater treatment sludge	Grigatti et al. (2019)
D2 mesophilic	Digestate	Operating scale	Liquid continous	na	-	Wine sludge	
BD	digestate	Operating scale	Liquid continous thermophilic	na	-	Bovine slurry and energy crops	

Table 2: Physico-chemical characteristics of the investigated digestates

Parameters	*Units*	**Agri_1**	**Agri_2**	**Agri_3**	**Sludge_1**	**Sludge_2**	**FFMSW_1**	**FFMSW_2**	**BW_1**	**Centr_1**	**Centr_2**	**D1**	**D2**	**BD**
TS	*%*	17.1%	4.4%	14%	22.4%	59.1%	19.7%	53.3%	24.9%	26.4%	6%	3.94%	3.09%	5.05%
VS	*%TS*	70.2%	70.1%	87.0%	51.7%	48.5%	48.7%	41.8%	79.0%	82.2%	60.0%	58.2%	59.4%	68.3%
TOC	*g kg^{-1}*	382.90	404.71	454.83	283.35	258.01	279.30	232.55	438.64	438.17	320.53	344.2	397.8	515.0
COD	*g.kg^{-1}*	1104.00	1400.56	1235.50	679.00	726.00	712.09	536.00	1291.00	1245.00	1497.58	nd*	nd	Nd
TKN**	*g.kg^{-1}*	25.85	115.00	nd	40.36	26.40	22.20	13.61	29.79	23.33	75.67	nd	nd	nd
TKN***	*g.kg^{-1}*	27.98	**44.53**	17.72	43.06	26.61	17.78	15.39	30.89	28.62	**45.47**	47.8	44.9	43.0
C/N		13.68	3.52	25.67	6.58	9.70	15.71	15.11	14.20	15.31	7.05	7.2	8.9	12.0
NH_4^+-N**	*g.kg^{-1}*	6.28	87.85	2.99	10.80	0.92	10.5	0.04	27.68	6.81	34.74	nd	nd	nd
P	*g.kg^{-1}*	5.36	10.37	4.00	20.44	16.15	4.21	4.23	10.79	6.93	17.98	7.1	18.4	6.2
K	*g.kg^{-1}*	21.22	27.41	12.98	2.15	4.90	11.49	8.22	7.25	4.86	16.10			
S	*g.kg^{-1}*	3.59	6.23	1.63	6.23	5.33	2.22	5.00	2.16	6.27	7.58		nd*	
Al	*g.kg^{-1}*	2.31	3.23	0.65	6.66	12.56	8.68	2.73	8.46	2.68	9.33			
Ca	*g.kg^{-1}*	18.53	23.08	13.44	69.08	41.25	37.05	29.72	42.31	17.56	27.08	10	37	11
Fe	*g.kg^{-1}*	1.58	2.81	0.94	42.60	37.48	5.79	5.06	7.89	11.54	12.07	1.9	8.6	1.0
Ca/P		0.29	0.27	0.24	2.08	2.32	1.38	1.19	0.73	1.67	0.67	1.4	2.0	1.7
Mg	*g.kg^{-1}*	5.49	6.28	1.56	3.52	3.72	3.24	4.29	3.47	1.98	5.13	2.8	11.6	5.2
References						This study						Grigatti et al. (2019)		

* nd : Not determined

** Measured on raw samples

*** Measured on from freeze-dried samples

Table 3: Dry biomass (DW), total P uptake and N uptake in ryegrass shoots during three successive harvests (day 28-84) and roots at the final harvest (day 84)

Treatment	Days after sowing						
	Shoots					Roots	Total
	28	56	84	*Mean*	0-84	84	84
DW (g pot^{-1})							
Ctrl-	0.87	0.53	0.25	*0.55*	1.65	1.25	2.90
Ctrl+	1.00	0.70	0.39	*0.70*	2.09	1.56	3.65
Agri_1	0.88	0.50	0.29	*0.56*	1.67	1.51	3.18
Agri_2	0.86	0.58	0.35	*0.60*	1.79	1.23	3.02
Agri_3	0.84	0.44	0.27	*0.52*	1.56	1.82	3.38
Sludge_1	0.94	0.58	0.42	*0.65*	1.94	1.55	3.49
Sludge_2	0.94	0.57	0.37	*0.63*	1.88	1.87	3.75
FFMSW_1	0.85	0.58	0.36	*0.60*	1.79	1.91	3.70
FFMSW_2	0.88	0.47	0.26	*0.54*	1.61	1.32	2.93
BW_1	0.99	0.53	0.41	*0.64*	1.92	1.79	3.72
Centr_1	0.89	0.54	0.37	*0.60*	1.81	2.30	4.11
Centr_2	0.96	0.58	0.41	*0.65*	1.95	1.28	3.23
P uptake (mg pot^{-1})							
Ctrl-	1.93	1.60	1.02	*1.52*	4.55	2.25	6.80
Ctrl+	2.17	1.48	1.43	*1.70*	5.09	2.24	7.33
Agri_1	2.41	1.14	1.61	*1.72*	5.16	2.87	8.03
Agri_2	1.81	1.15	1.76	*1.58*	4.73	2.19	6.92
Agri_3	1.87	1.07	1.76	*1.57*	4.70	2.86	7.56
Sludge_1	2.28	1.23	2.70	*2.07*	6.21	2.96	9.17
Sludge_2	2.29	1.53	2.32	*2.05*	6.14	3.18	9.32
FFMSW_1	2.05	1.76	2.41	*2.07*	6.21	2.62	8.84
FFMSW_2	2.65	1.13	1.35	*1.71*	5.13	2.22	7.35
BW_1	2.76	1.40	2.38	*2.18*	6.54	2.39	8.93
Centr_1	2.77	1.36	2.26	*2.13*	6.39	2.58	8.97
Centr_2	2.67	1.53	2.31	*2.17*	6.51	2.25	8.76
N (mg pot^{-1})							
Ctrl-	25.01	9.32	6.52	*13.62*	40.85	12.44	53.28
Ctrl+	45.70	16.69	9.23	*23.87*	71.62	14.89	86.51
Agri_1	25.29	7.35	8.21	*13.62*	40.85	14.76	55.61
Agri_2	29.41	9.03	8.98	*15.81*	47.42	13.31	60.73
Agri_3	21.84	6.48	6.80	*11.70*	35.11	14.71	49.82
Sludge_1	36.91	10.51	12.39	*19.94*	59.81	15.13	74.93
Sludge_2	35.32	9.72	10.52	*18.52*	55.55	17.49	73.05
FFMSW_1	28.77	10.34	9.67	*16.26*	48.78	15.35	64.13
FFMSW_2	29.80	8.97	7.41	*15.39*	46.17	15.45	61.62
BW_1	32.03	9.97	11.88	*17.96*	53.88	16.51	70.39
Centr_1	30.74	9.28	10.00	*16.67*	50.02	18.45	68.47
Centr_2	34.63	10.39	12.16	*19.06*	57.18	14.04	71.22

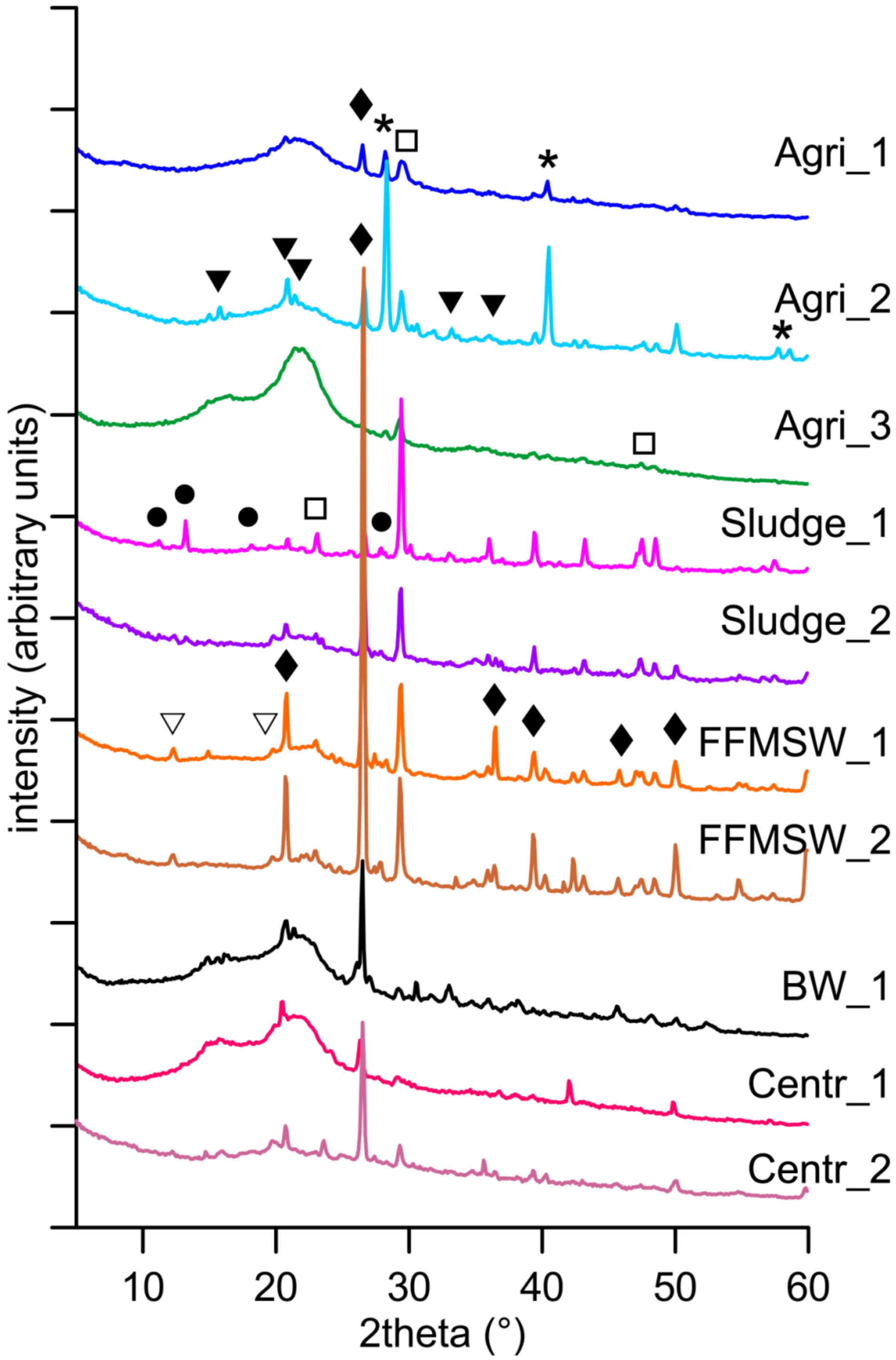
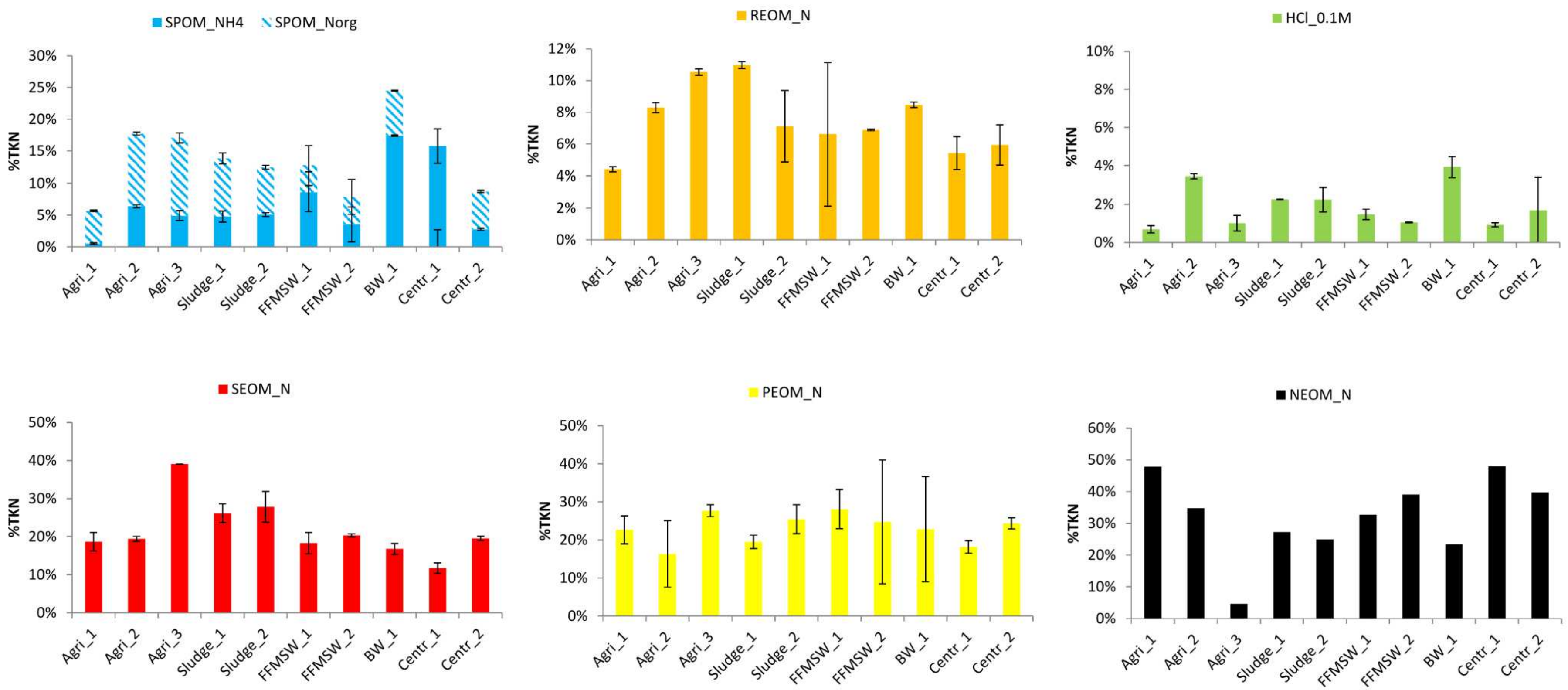
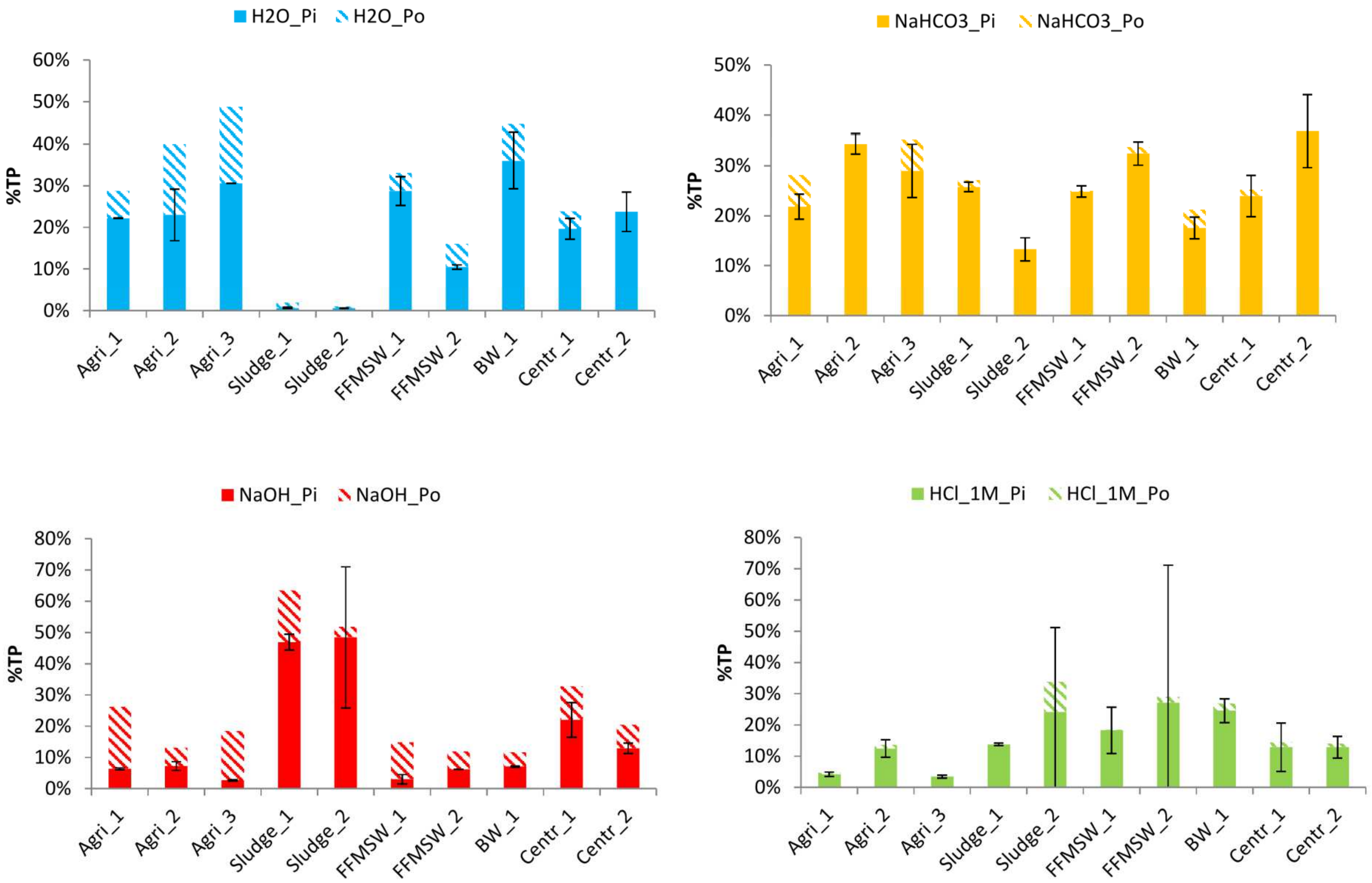


Figure 1: X-ray diffraction of the digestates. The main peaks of different crystalline phases are identified by symbols: ▼ struvite; ♦ quartz; □ calcite; ● vivanite; * sylvine; ▽ kaolinite.

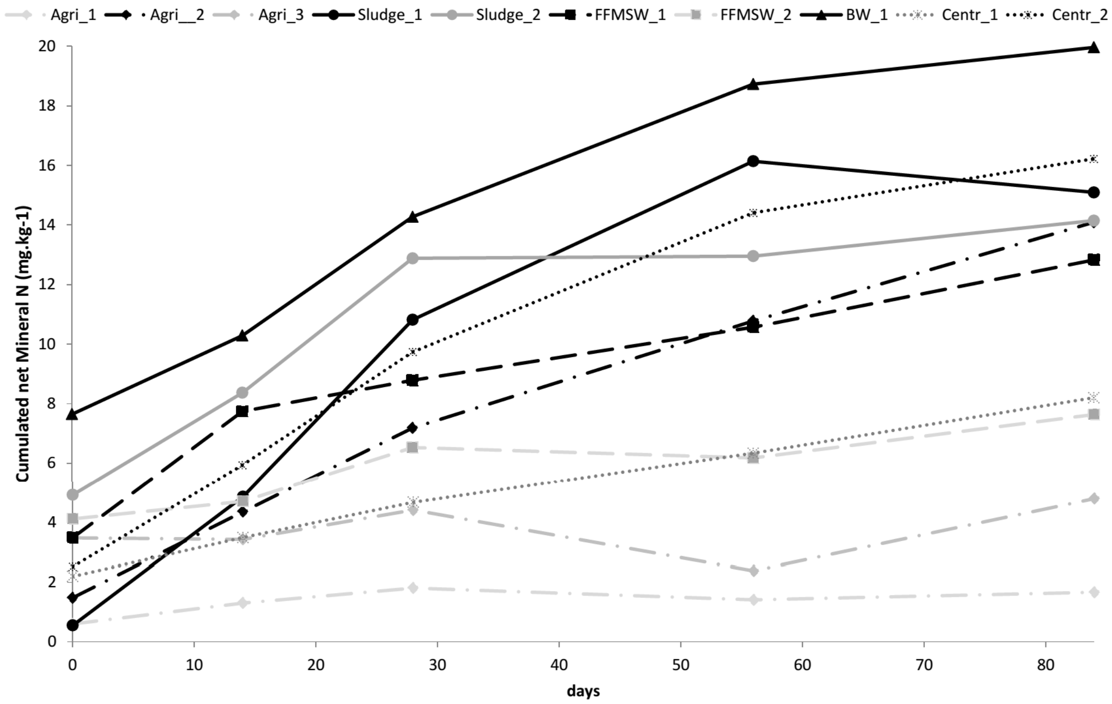

SPOM_NH4
SPOM_Norg
REOM_N
HCl_0.1M
SEOM_N
PEOM_N
NEOM_N
%TKN
Agri_1
Agri_2
Agri_3
Sludge_1
Sludge_2
FFMSW_1
FFMSW_2
BW_1
Centr_1
Centr_2

(a)

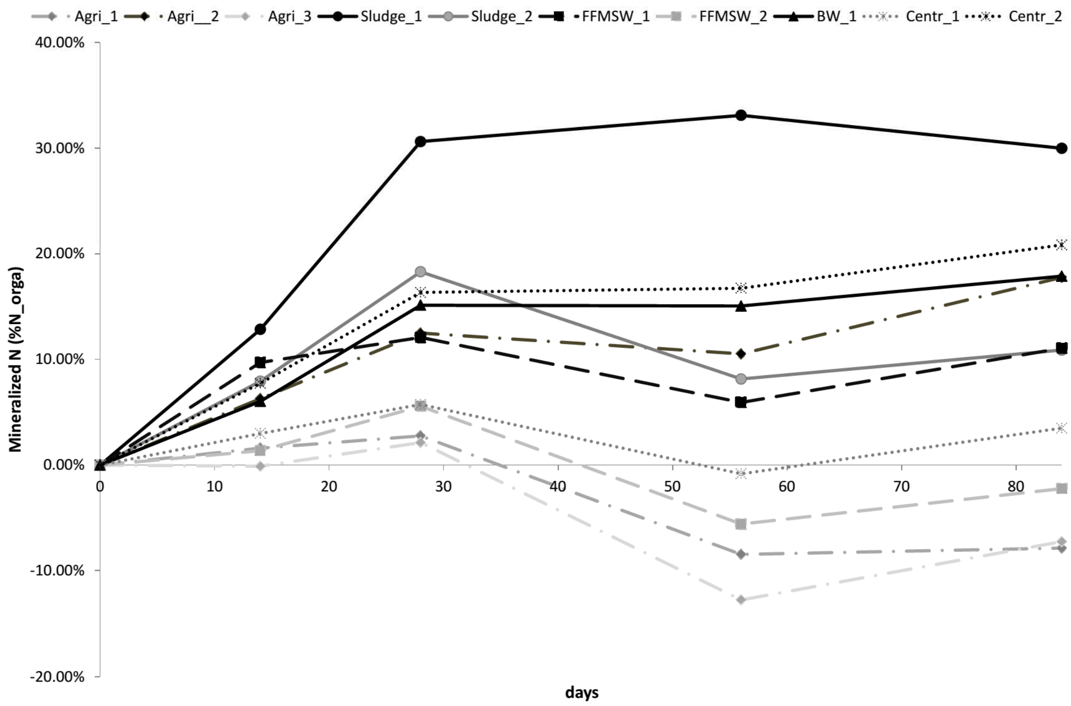


(b)

Figure 2: SCE fractions of TKN (a) and TP (b) obtained for all the digestates, according chemical accessibility. Error bars, SE (n=2)

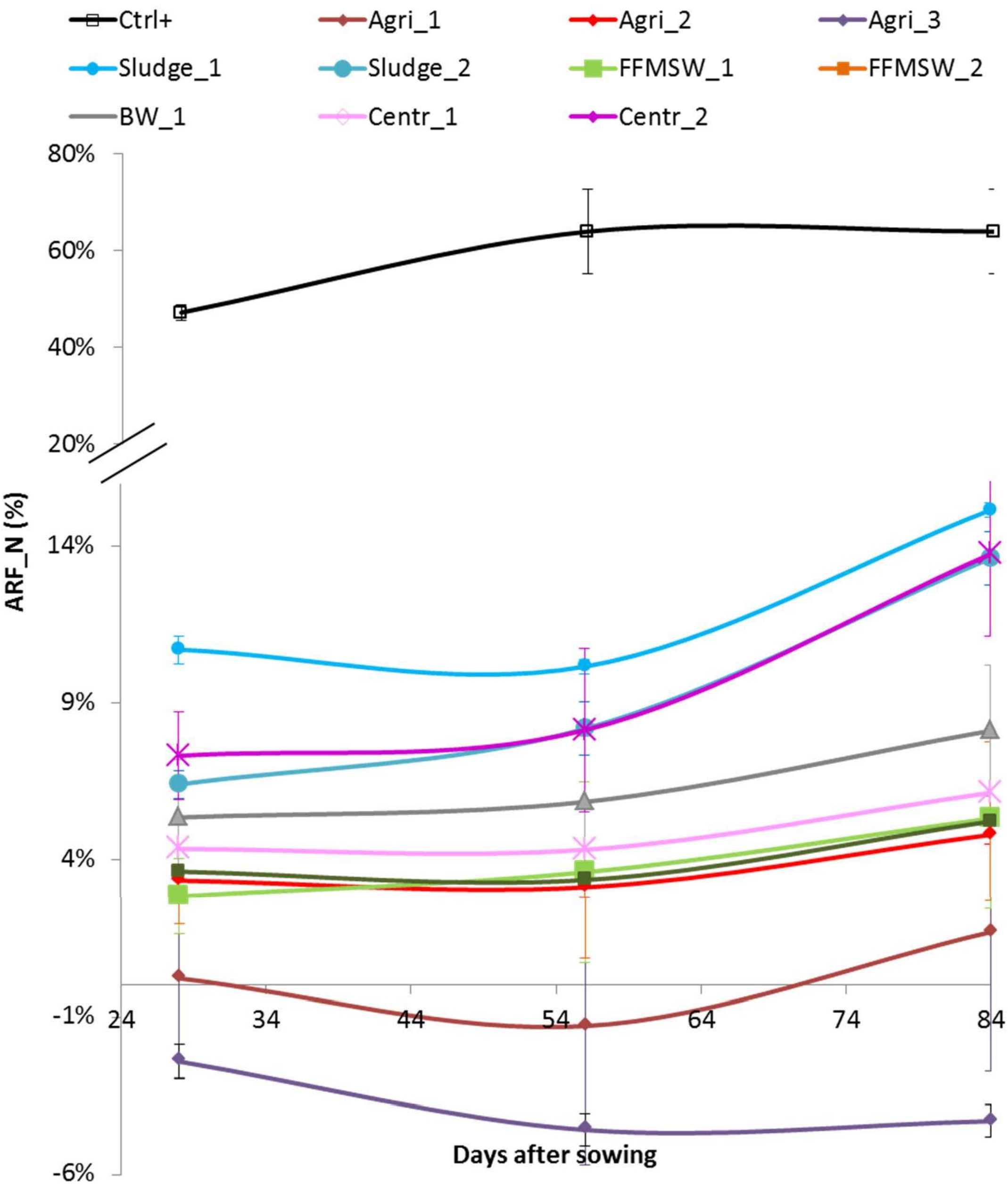


(a)



(b)

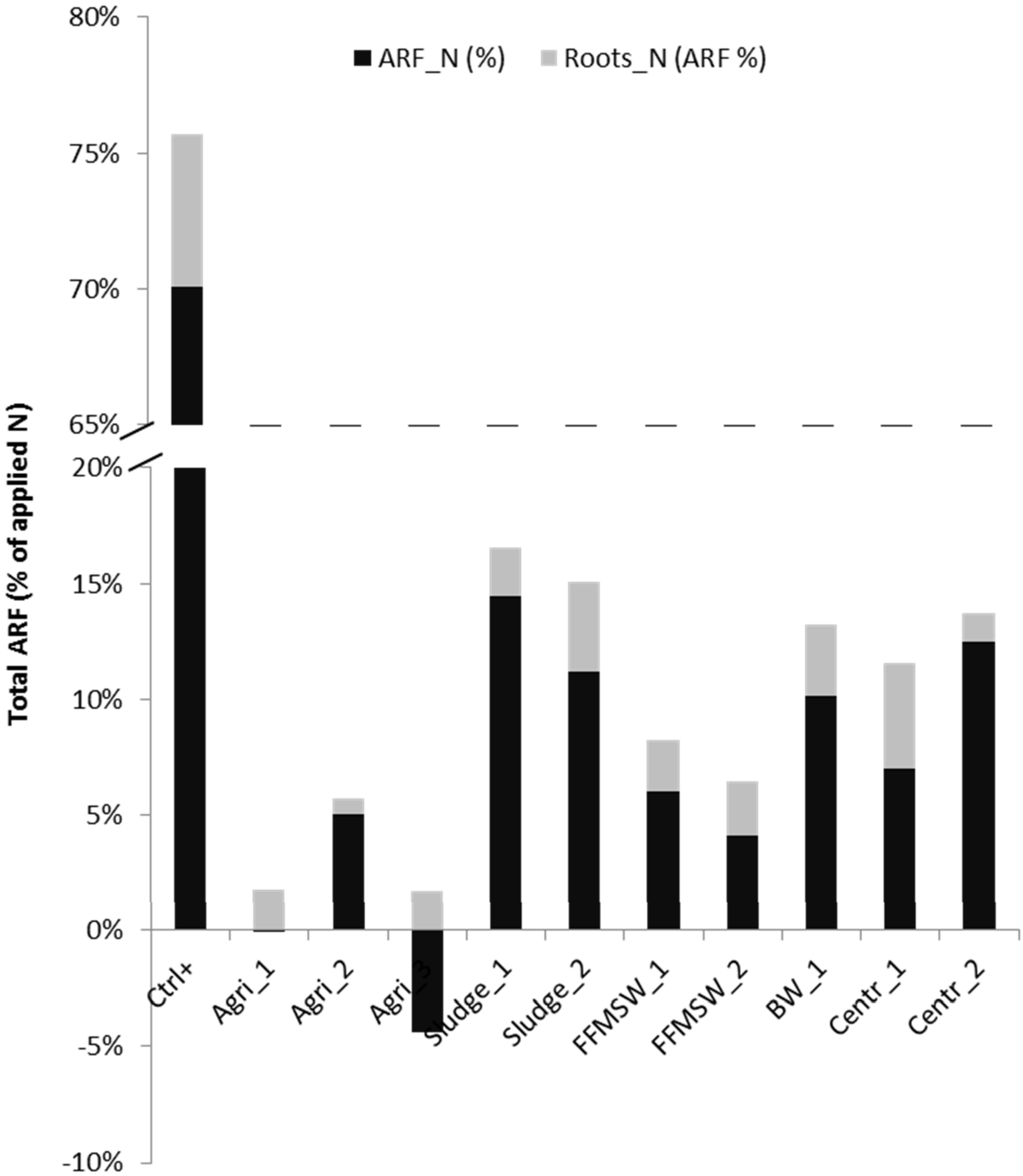
Figure 3: Cumulated mineral nitrogen concentration evolution (a) and cumulated mineralized organic nitrogen percentage (b) during soil incubation. Net values. *Black: high mineralized N group; Grey: low mineralized N group (circle+full line: sludge digestates, square+large dashes: FFMSW digestates, diamond+ dashes and dots line: Agrowastes digestates, triangle: biowaste digestate, cross+dotted line: centralised digestates)*



Ctrl+
Agri_1
Agri_2
Agri_3
Sludge_1
Sludge_2
FFMSW_1
FFMSW_2
BW_1
Centr_1
Centr_2
80%
60%
40%
20%
14%
9%
4%
-1%
-6%
ARF_N (%)
24
34
44
54
64
74
84
Days after sowing

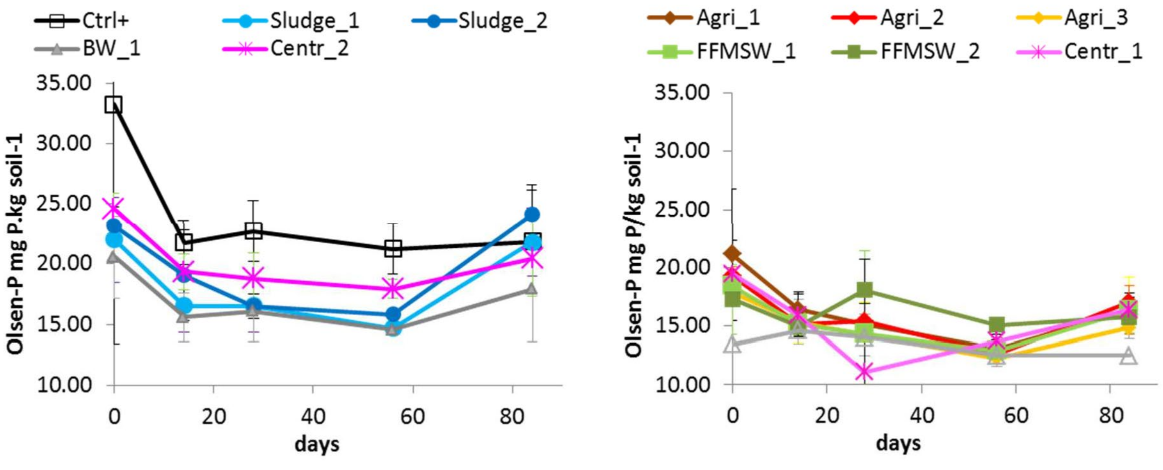
(a)

(a)

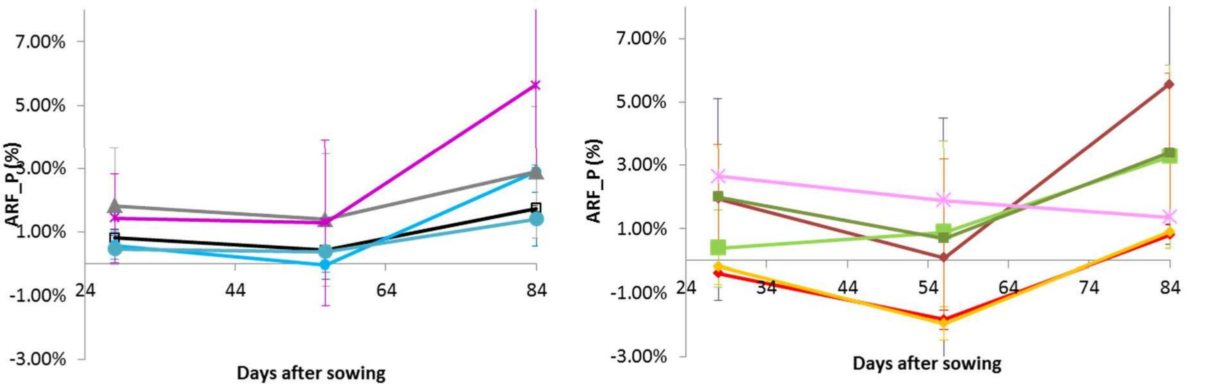


(b)

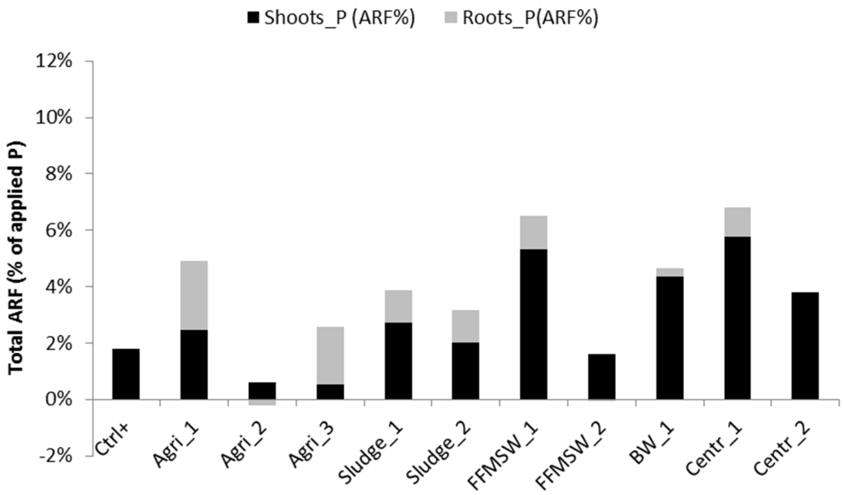
Figure 4: Nitrogen apparent recovery fraction (ARF) of ryegrass shoots (a) and in both shoots and roots (b) in three harvests during pot trial. Error bars, SE (n=3)


Ctrl+
Sludge_1
Sludge_2
BW_1
Centr_2
Olsen-P mg P.kg soil-1
35.00
30.00
25.00
20.00
15.00
10.00
0
20
40
60
80
days
Agri_1
Agri_2
Agri_3
FFMSW_1
FFMSW_2
Centr_1
Olsen-P mg P/kg soil-1
35.00
30.00
25.00
20.00
15.00
10.00
0
20
40
60
80
days

(a)



(b)



(c)

Figure 5: Olsen-P evolutions during the soil incubation of the tested digestates (a), cumulative