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Chapter 8

Biomass Digestion to Produce Organic Fertilizers: A Case-Study on Digested Livestock Manure

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53869

1. Introduction

Biogas production by anaerobic digestion of organic wastes coming from agricultural practices is one of most promising approach to generate renewable energy, giving as end-product a digested organic biomass with specific characteristics useful for soil fertilization. This last aspect represents an opportunity in relation to the need to close the nutrient cycles within the agricultural and natural ecosystems, particularly in specific systems underwent to a constant resources depletion, as those of Mediterranean area, where the C-sink loss represents one of the main causes of desertification [1], [2]. The composting process was yet identified as one of the promising answers to the need of soil organic matter conservation, such as the addition to the soil of different organic materials of different origins [3], but the anaerobic digestion could represent an effective further step able to guarantee the recycle of nutrients, coupled with an environmental-friendly energy production [4].

Particularly, anaerobic digestion of livestock manures allows us to achieve several purposes: i) renewable energy generation; ii) reduction of nitrate leaching in livestock exploitations, iii) production of an organic biomass as by-product employable as organic fertilizer [5]. Actually, digestates coming from livestock manure give biomasses characterized by biologically stable organic matter and relevant nitrogen content; these traits suppose that these biomasses may be usefully utilized as N-fertilizers and soil organic amendments in agriculture, but also as a component of growing media in pot horticultural cultivation.

It should be remarked that production of greenhouse horticultural crops requires the use of growing media of high quality, with specific physical-chemical characteristics. Being the peat, organic component traditionally used in substrates formulation, a non-renewable resource (its extraction involving many environmental issues), it becomes ever more urgent the need to individuate alternative organic materials with the same functioning, such as composts or products coming from biogestion processes [6]. Nevertheless, the use of

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biodigestate as amendment, still now not allowed in Italy, should provide the declaration of stability parameters for organic matter, since the utilization of matrices not properly stabilized could lead to the risk of high fermentescibility of organic components and, thus, consequent phytotoxicity phenomena [6],[7]. The stabilization of organic matter actually involves the mineralization of the most labile organic fraction, with the following decrease of C/N ratio; this means physical, chemical and biological changes of the starting material and, thus, the decrease of porosity, increase of pH, CEC, bulk density and salinity, due to a concentration of organic compounds which, generally, are characterized by lower molecular weight respect to the starting ones, more resistant to microbial degradation [8].

The amendment properties of composts and biodigestates could be assessed by different analytical methods, such as the isoelectrophoretic techniques (IEF) [9],[10],[11]. Results obtained after IEF characterization of the extractable fraction in alkaline environment of dried vine vinasse (an anaerobically digested solid residues, constituted by exhausted stems, skins and grape seeds, obtained after distillation of the "grappa", the Italian "acquavite"), showed an increase of the extractable organic components in alkaline environment, with a higher content in less acidic organic fraction, probably due to a "concentration effect" of the more complex organic components not, or only partially, degraded during the anaerobic digestion process.

Other works demonstrated also that the same dried vine vinasse, applied together with other mineral components in growing media, was able to increase nutrient availability [12] and express a sort of biostimulant activity on plant roots [13]. Study performed by optical microscopy demonstrated that digested vine vinasse in combination with clinoptilolite addition, promoted maize roots development, by increasing mucigel production by root tip and thus favoring the following solubilization and uptake of nutrients by plant from the added organic biomass (Figure 1).

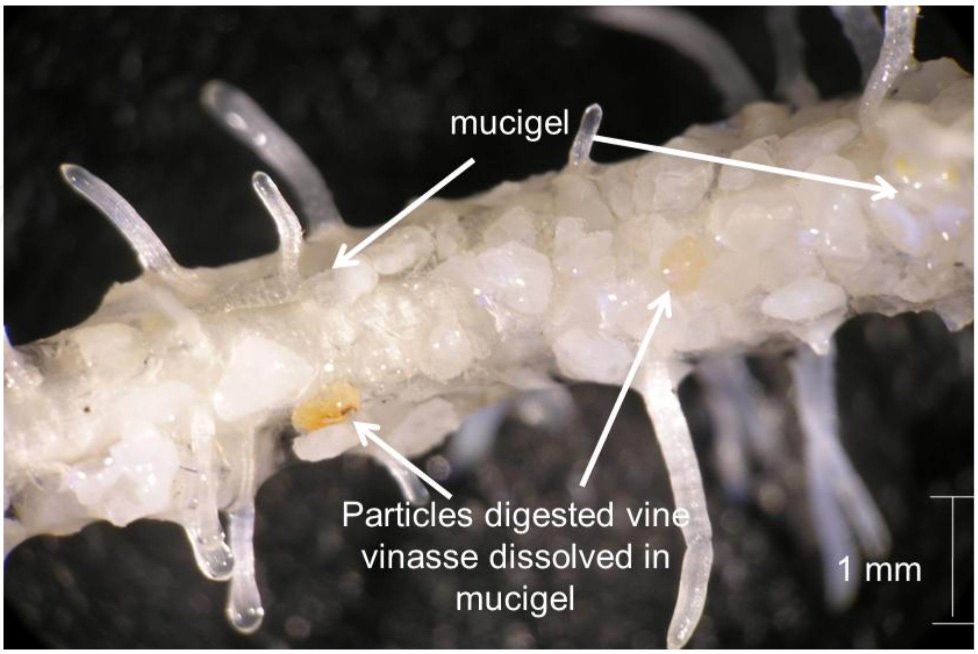


Figure 1. Root of Zea mais L., treated with micronized clinoptilolite and digested vine vinasse.

In relation to the N-fertilizer attitude of a biodigestate, it has a particular relevance in the case of the biodigestion of animal manure. The anaerobic process allows to maintain constant the total N amount of the original material, even if the organic N is mainly transformed into ammonia, the mineral form most easily available to the crops. The separation between the liquid and the solid fractions after biodigestion allows to recover the ammonium-N in the fluid fraction and the residual organic matter in the solid fraction, so to emphasize the different characteristics of the two fractions: the first one, usable as a typical N-fertilizers, able to furnish nutrient supply to plant; the second one as organic amendment, able to supply organic matter to soil and then improve its chemical, physical and biological characteristics. A proper composting process applied to the solid fraction of this digestate could lead to a further improvement of the biomass, by promoting its biochemical stability and giving those amendment properties yet described, which constitute the adding value of the final product.

What is relevant is that the risk of nitrate leaching in water represents the main limitation to the direct application of not pre-treated livestock manure to soil: effectively, its amendment properties, linked to soil organic matter addition, often go in conflict with the Council Directive 91/676/EEC on the “Water protection from nitrates” [14], especially in vulnerable zones, such as those of Italian northern regions. In this sense, the biodigestion process represents a great opportunity to utilize livestock manure digestates as N fertilizer, potentially allowing to overcome the limit of 170 Kg N ha^{-1} year^{-1} superimposed by EU Nitrates Directive, since the high N-efficiency coefficient of these digestates, similar to that of soluble mineral fertilizers [15]. The solid fraction, containing organic matter already partially stabilized, could also permit its application during the winter season, provided the obtained amendment has a constant composition and a fraction of slow release-N, eventually increased by a following composting process [16],[10],[17]. On the other hand, the anaerobic conditions ensure the formation of high amount of ammonium during the organic matter degradation process, without incurring in the subsequent oxidation into nitrate [18],[4]. Being the ammonium the N-form more rapidly assimilated by the crops, this could be a further element in favor of the utilization of biodigestates as components of growing media, effectively conjugating the physical amendment properties with those chemical, connected with fertilization.

In 2007, the Italian ministry of agriculture financed a Research Project on the “Anaerobic digestion of livestock manure and EU Nitrates Directive – Effect due to the anaerobic digestion on N availability in livestock manure for overcoming the limit of 170 Kg N ha^{-1} year^{-1} superimposed by imposto EU Nitrates Directive”. The main aim of the reported study was to verify the N-fertilizer properties of a digestate coming from a swine livestock manure, taking into account the possibility to utilize this processed agricultural waste of animal origin for limiting the risk of environmental pollution. Hereafter, results related to the effect of the digested and not-digested solid fraction of this bovine livestock manure applied as N-organic fertilizers on lettuce growth in a greenhouse experiment are reported.

2. Materials and methods

2.1. Soils characterization

Two different soils of Northern Italy were chosen in a specific area located in Pedemont Region (Vercelli, Italy) characterized by cold and humid climate, in relation to their recognized vulnerability for nitrate leaching. The two soils, Tetto Frati (A) and Poirino (B), sampled at 0-30 cm, were characterized for main chemical, physical and biochemical parameters: pH, CEC (meq 100 g^{-1}), total organic C % [19] and total N (mg Kg^{-1}) [20], texture (sand %, loam %, clay %), bulk density (g cm^{-3}), basal respiration [(mg_{C-CO2} ×Kg_{soil}^{-1})×day^{-1}], C microbial biomass content (mg kg^{-1}) [21], metabolic quotient qCO_2 [(mg_{C-CO2}×mg C_{biom}^{-1}×h)×10^{-3}], mineralization quotient qM, [22] and C microbial biomass/C organic ratio (%) [23].

2.2. Biomasses characterization

The organic biomasses, not-processed and processed, were supplied by the anaerobic digestion plant of the "Research Centre for Animal Production - Foundation Centre for Studies and Research" (C.R.P.A. – F.C.S.R. S.p.A., Reggio Emilia, Italy). Their main chemical characteristics of solid fractions of swine livestock manure are below reported:

pH:	not-digested = 7.0	digested = 8.0
N content (as received):	not-digested = 0.47%	digested = 0.39%
N content (dry matter)	not-digested = 2.9%	digested = 3.0%
C organic (dry matter):	not-digested = 43,5%	digested = 35,9%

In order to evaluate the organic matter stability of the two biomasses, both the digested and not-digested solid fractions of swine manure were previously analyzed by isoelectrofocusing (IEF) technique. Organic matter extraction was carried out on 2 g of each biomass with 100 mL of a solution NaOH/$Na_4P_2O_7$ 0.1N for 48 hours at 65°C. After centrifugation and filtration, the extracts were stored at 4°C under nitrogen atmosphere. Ten millilitres of NaOH/$Na_4P_2O_7$ extracts were dialysed in 6,000-8,000 Dalton membranes, lyophilised and then electrofocused in a pH range 3.5-8.0, on a polyacrylamide (acrylamide/bis-acrylamide: 37.5/1) slab gel, using 1 mL of a mixture of carrier ampholytes (Pharmacia Biotech) constituted by: 25 units of Ampholine pH 3.5-5.0; 10 units of Ampholine pH 5.0-7.0; 5 units of Ampholine pH 6.0-8.0. A prerun (2h 30′; 1200V; 21mA; 8W; 1°C) was performed and the pH gradient formed in the slab gel was checked by a specific surface electrode. The electrophoretic run (2h; 1200V; 21mA; 8W; 1°C) was carried out loading the same C amount of water-resolubilised extracts (1 mg C×50 L^{-1} × $sample^{-1}$). The bands obtained were stained with an aqueous solution of Basic Blue 3 (30%) for 18 h and then scanned by an Ultrascan-XL Densitometer (Amersham – Pharmacia) [10].

2.3. Pot trial on lettuce (experimental plan, plant biometric survey and elemental analysis)

In a 300 m^2 greenhouse, lettuce (Lactuca sativa L., var. Romana) were transplanted into 2 L and 16 cm diameters pots, containing the A and B soils; growing density was 16 pots m^2. The experiment was performed from April to June, 2009, at temperatures range of 15-28°C.

Treatments consisted in a factorial combination of two increasing N doses (200 and 400 $kg_N \times ha^{-1}$), applied as solid fraction of digested swine livestock manure, not-digested solid fraction of swine livestock manure and granular urea [$CO(NH_2)_2$], taken as conventional mineral fertilization; not-fertilized soils were also considered as control treatments. Even if the N recommended dose for lettuce growth is about 90-100 $kg_N \times ha^{-1}$, the choice of such high N supply was made on the basis of the need to overcome the limit of 170 Kg_N ha^{-1} by substituting these organic biomasses rich in N to the mineral fertilization, without incurring in undesired effects on plant and environment: the dose of 400 $kg_N \times ha^{-1}$ was just applied for evaluating its potential phyto-toxicity on lettuce in relation to the different fertilization treatments. The corresponding fertilizers'doses per pot were: 128.6 g and 257.2 g for ND, 153.2 g and 306.3 g for D and 1.4 g and 2.6 g for urea.

Treatments were arranged in a randomized complete-block design with six replicates. Drip irrigation was managed in relation to plant water-demand, as reported in Figure 2.

Figure 2. Example of pot cultivation of lettuce in greenhouse; drip irrigation was used for guaranteeing daily water supply to the plants .

Lettuce plants were harvested 70 days from sown; biomass dry weight (g), dry matter (%), leaf area (cm^2) and leaf number were determined for each plants. In order to evaluate the effect of alternative fertilization treatments on micro and micronutrient uptake by lettuce, N, P, K, Mg, Cu, B, Fe, Mn leaf contents, plant material was incinerated at 400°C for 24 hours; ashes were then redissolved in HCl 0.1N and the supernatant filtrated to obtain a limpid solution; the nutrient content was determined by simultaneous plasma emission spectrophotometer (ICP-OES) on obtained solution and calculated in relation to dry matter.

2.4. N use efficiency

After analysis of N leaf tissue content (%) by Kijeldhal method, N-use efficiency (NUE %) was calculated, as the percentage of the N uptaken by the lettuce plant respect to N supplied by the fertilizer.

In order to study the long-term effect of the alternative fertilization approaches, the soil residual N at the end of experiment was obtained after Kjeldhal digestion and titrimetric determination [20]. Then, the available $N\text{-}NO_3$ and exchangeable $N\text{-}NH_4$ in the soils were determined after extraction of 4 g of each soil in 40 mL of KCl 0.2 N solution and subsequent colorimetric analysis of the supernatant by Automatic Analyzer Technicon II.

2.5. Statistical analysis

Plant biometric and soil N data were evaluated by ANOVA to verify the statistical differences of the tested parameters in relation to the different fertilization treatments.

Elemental data were analysed using vector analysis, which allows the simultaneous evaluation of plant dry weight and nutrients content in an integrated graphic format [24],[25]. Elemental data in relation to the different treatments in soils A and B were normalized with respect to urea al 200 kg_N ha^{-1}, taken as reference treatment.

3. Results and discussion

3.1. Soils characterization

In Table 1, the chemical-physical and biochemical parameters of the two considered A and B soils are reported.

The comparison between soils characteristics showed that A was a typical sandy-loam soil, with a lower organic C, lower C microbial biomass content, lower C microbial biomass/C organic ratio and higher mineralization quotient respect to B, which was a loamy soil, with higher organic C, higher C microbial biomass content, higher C microbial biomass/C organic ratio and lower mineralization quotient (Figure 1). On the basis of these results, the two soils were defined as a low biological fertility soil (Soil A) and a medium biological fertility soil (Soil B) [[23],[26].

The choice of these two different soils was performed in order to evaluate the effect played by the different soil characteristics on the behaviour of the digested and not-digested materials utilized in the experiment: it is well known that in all biologically mediated processes, such as the mineralization/immobilization of N coming from organic fertilizers, the microbial biomass has a key-role in addressing the degradation of organic substrate and making available N to the plant [27],[28],[29].

On the basis of what above discussed, the results were elaborated by considering the two soils as two independent experimental units, where the same experimental design was applied.

Parameter	Soil A	Soil B
Sand (%)	48,4	15,8
Loam (%)	43,1	75,6
Clay (%)	8,5	8,6
pH	8,2	6,1
C organic (g kg^{-1})	12	17
N total (mg kg^{-1})	0,83	0,81
CEC (meq $100g^{-1}$)	8,2	12,5
Bulk density (g cm^{-3})	1,34	1,45
Basal respiration (mg $_{C\text{-}CO2}$ Kg_{soil}^{-1}) day^{-1}	7,6	5,7
C microbial biomass (mg kg^{-1})	172	281
C microbial biomass/C organic (%)	1,43	1,65
qCO_2 (mg $_{C\text{-}CO2}$ mg $_{Cbiom}^{-1}$ h) 10^{-3}	1,85	0,85
q*M* (%)	4,3	3

Table 1. Main chemical, physical and biochemical parameters of A and B soils.

3.2. Biomasses characterization

In Figure 3, isoelectric focusing of the extracted organic matter from not-digested and digested solid fraction of swine manure utilized in the experimental activity is reported.

As reported in literature [30],[31], the more humified is the organic matter, the less acidic and higher in molecular weight are the organic compounds which compose it. This information, which is valid for soil humic matter but also for humic-like compounds in biomasses, allows to use IEF technique in order to separate organic matter extracted from different materials. Taking into account that, generally, organic matter focused in a pH range between 3.0 and 5.5, obtained results indicated that the organic matter from digested solid fraction was better stabilized respect to the not-digested one: in fact, the increasing peaks at pH >4.7 indicated a good stabilization of extracted organic matter from digested material, that means increased amendment properties of this material [16].

Effectively, the increase of the peaks'area in the pH region higher than 4.5 indicates firstly, that during the biodigestion changes in chemical composition of the organic material took place and, secondly, that these transformations led to the constitution of a final biomass made by less acidic compounds, higher average molecular weight molecules, that means more chemically stable material [10].

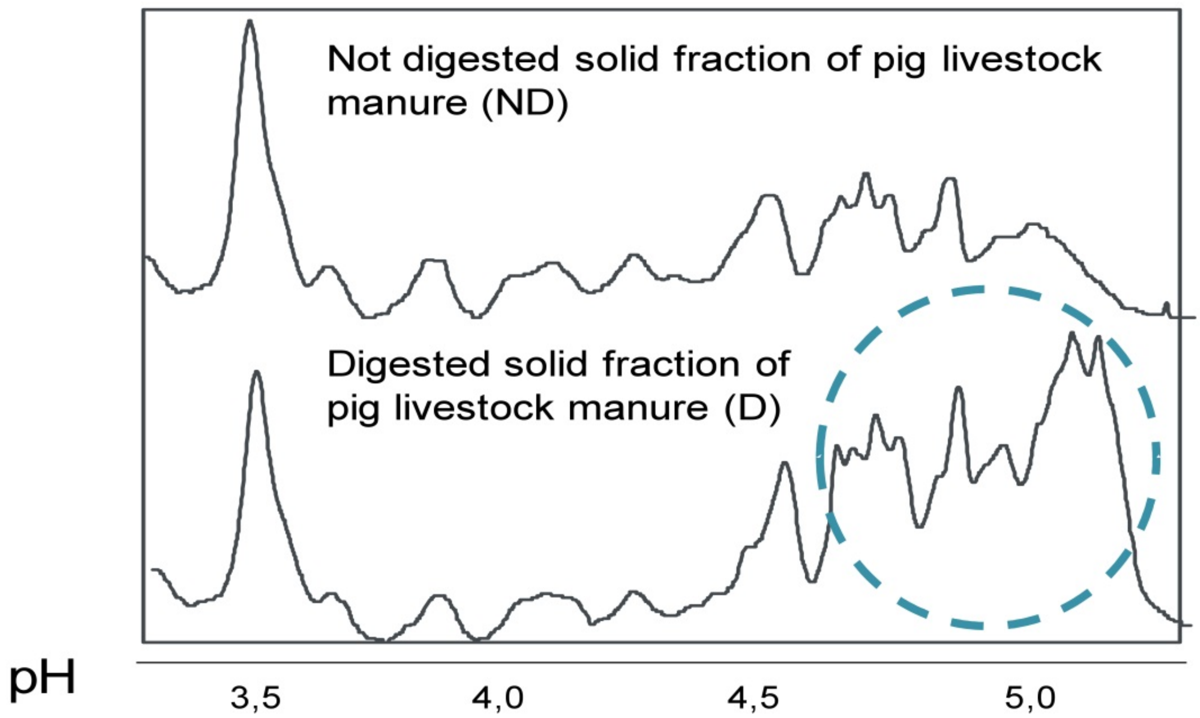


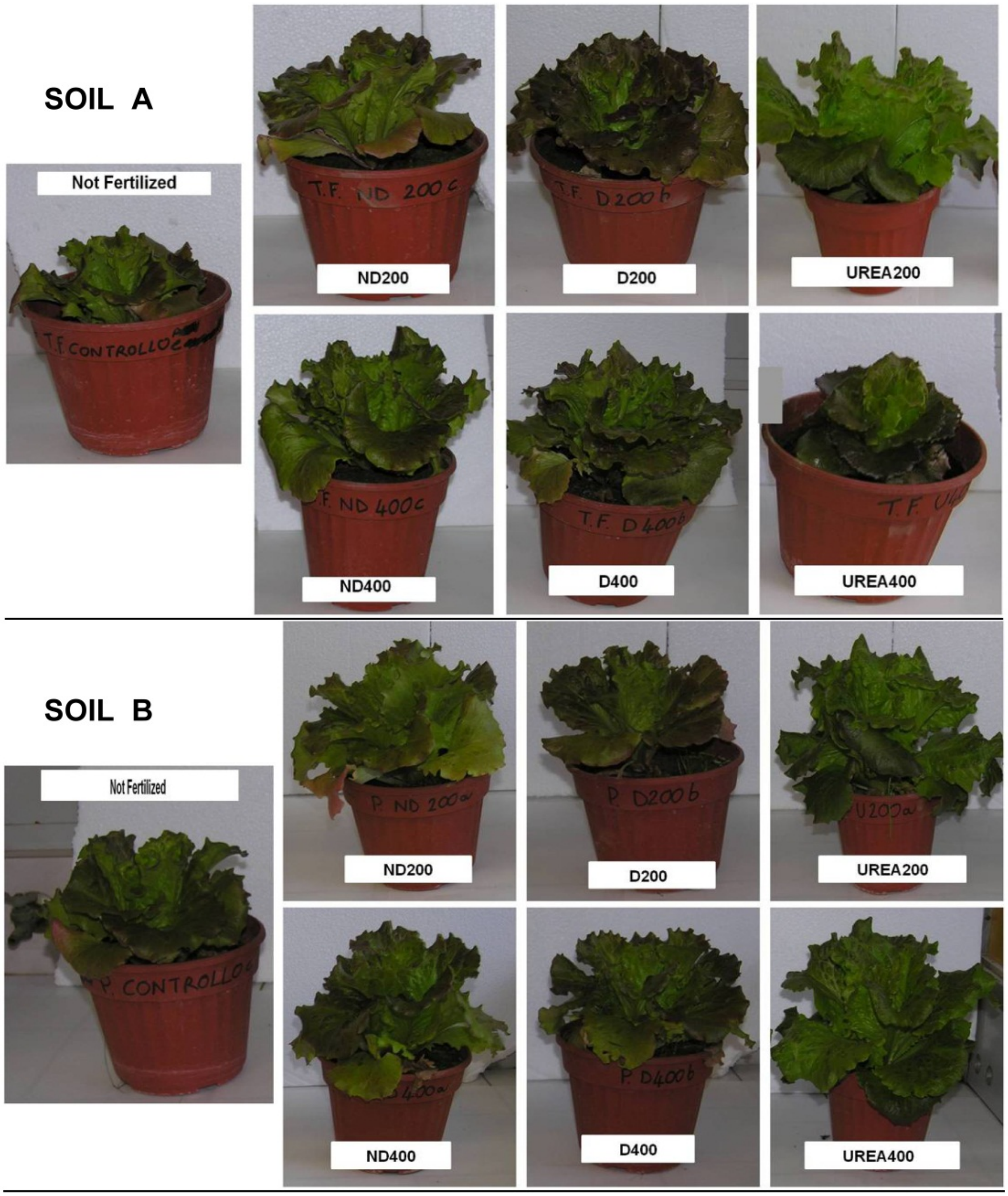
Figure 3. Isoelectrofocusing of extracted organic matter in alkaline environment from not-digested and digested solid fraction of pig livestock manure.

3.3. Pot trial on lettuce (plant biometric survey and elemental analysis)

In Figure 4, lettuce plants differently fertilized in the two soils at the end of the experiment (60 days) are shown.

The effect of the different treatments and soils is evident when comparing the lettuce growth. The phytotoxic effect of urea at highest dose was dramatically shown in Soil A, while at the same urea dose, in soil B the lettuce was able to increase its development although the excess of N mineral supply: this result attested clearly the role of soil in influencing the actual availability of nutrients, and in particular of N, for plant uptake. It was promising the good performances of both the not-digested and the digested solid fraction of livestock manure at the highest N dose, particularly in Soil A: even if the 400 $kg_N \times ha^{-1}$ supplied by urea gave the worst result on lettuce, apparently the excess of N added with the organic biomasses did not determine any decrease of lettuce growth, but on the contrary, a very good development of lettuce foliage (Figure 5).

This aspect is a positive point in order to propose the increase of the limit rate of 170 $kg_N \times ha^{-1}$ $year^{-1}$ for digestate application to soil, especially because these results were obtained in a soil with a sandy texture, low organic C content and, consequently, particularly vulnerable for nitrates.



(ND = not-digested solid fraction of livestock manure; D = digested solid fraction of livestock manure; 200 = 200 $kg_N \times ha^{-1}$; 400 = 400 $kg_N \times ha^{-1}$).

Figure 4. Lettuce grown in relation to the different fertilization treatments in soil A and B.

(400 = 400 $kg_N \times ha^{-1}$).

Figure 5. Example of lettuce leaves development in relation to the different fertilization treatments in Soil A.

The quantitative results related to biomass production and quality of plants are reported in Table 2.

Soil A	Dry weight (g plant^{-1})	Dry matter (%)	Total leaf area (cm^{-2})	Number of leaves
Not fertilized	1,4 b	5,2 b	450 b	15 a
Urea 200 Kg ha^{-1}	2,9 d	4,8 b	1450 d	24 c
Urea 400 Kg ha^{-1}	0,4 a	5,4 c	180 a	13 a
Not digested 200 Kg ha^{-1}	1,2 b	4,4 a	730 c	17 b
Not digested 400 Kg ha^{-1}	2,5 c	7,3 d	750 c	18 b
Digested 200 Kg ha^{-1}	1,9 c	5,0 b	760 c	17 b
Digested 400 Kg ha^{-1}	2,4 c	5,0 b	850 c	18 b
Soil B	**Dry weight (g plant^{-1})**	**Dry matter (%)**	**Total leaf area (cm^{-2})**	**Number of leaves**
Not fertilized	0,7 a	1,9 a	900 b	18 ab
Urea 200 Kg ha^{-1}	3,5 c	3,7 c	2000 c	23 c
Urea 400 Kg ha^{-1}	0,8 a	5,2 cd	1300 bc	22 c
Not digested 200 Kg ha^{-1}	1,7 b	3,7 c	850 b	18 ab
Not digested 400 Kg ha^{-1}	1,6 b	3,2 b	1250 bc	20 b
Digested 200 Kg ha^{-1}	0,7 a	2,1 a	700 a	17 a
Digested 400 Kg ha^{-1}	1,6 b	3,5 c	1000 b	20 b

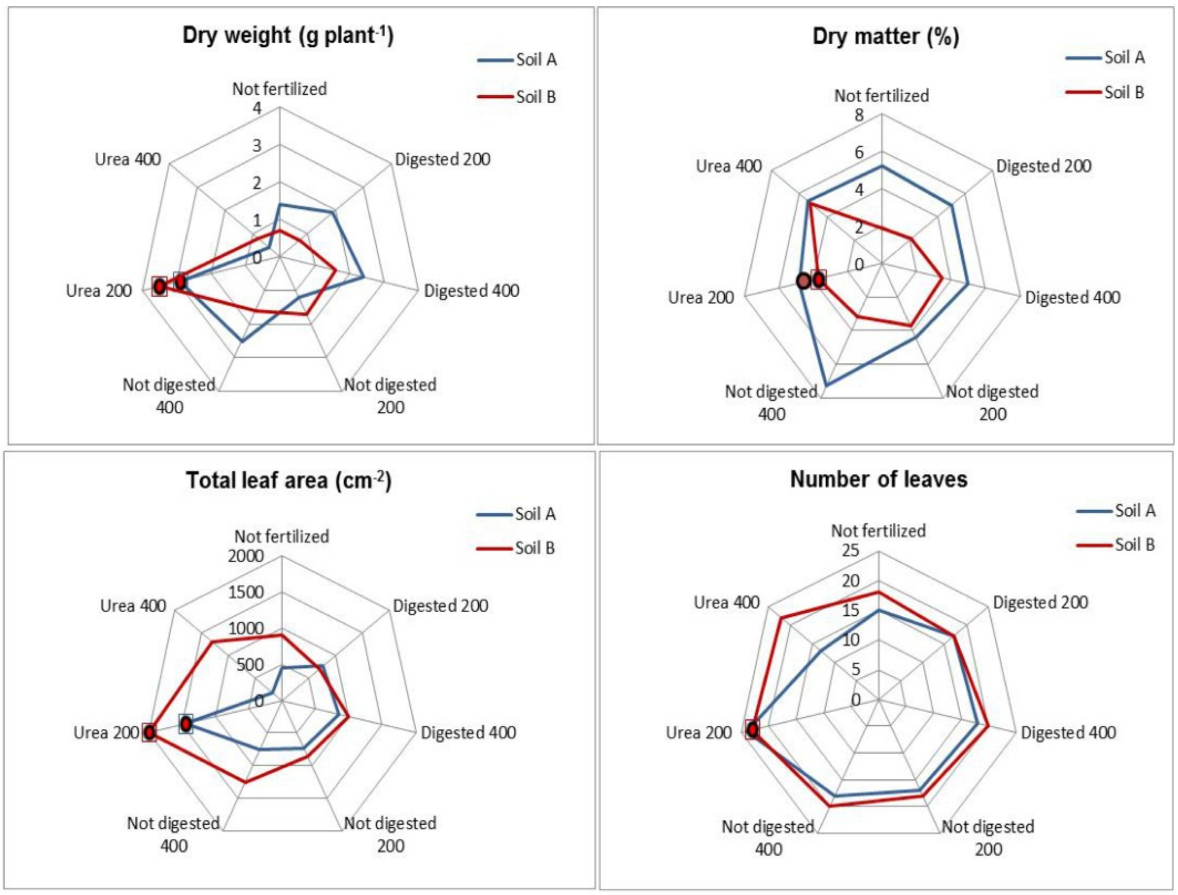
Table 2. Lettuce dry weight, dry matter, total leaf area and number of leaves obtained after fertilization treatments in A and B soils (average value; different letters means significant differences at P-level<0.05).

Firstly, again a strong "soil effect" was recorded in relation to plant growth parameters for all the treatments, due to the different chemical-physical characteristics and biological fertility of the two soils. Not fertilized plants showed a limited vegetative development while, as expected, lowest urea dose (200 $kg_N \times ha^{-1}$) gave the best plant growth (6.7 g $plant^{-1}$), as confirmed by recorded parameters as shoot dry weight, percentage of dry matter, leaf area and leaf number, especially in B Soil. On the contrary, urea at 400 $kg_N \times ha^{-1}$ dramatically depressed plant growth in Soil A (0.8 g $plant^{-1}$), due to evident toxicity phenomena.

Digested and not digested biomasses gave best results when applied at the higher dose respect to the lowest one; actually, in treatments with both the biomasses at 400 $kg_N \times ha^{-1}$, plant parameters were closer to those obtained with urea at 200 $kg_N \times ha^{-1}$. It is relevant that in Soil A the application of both the digested and the not-digested solid fractions of livestock manure at 400 $kg_N \times ha^{-1}$ gave weight parameters higher than those observed at 200 $kg_N \times ha^{-1}$. Otherwise, in Soil B, only fertilization with digested solid fraction of livestock manure at 400 $kg_N \times ha^{-1}$ gave an increase of all the tested parameters respect to the 200 $kg_N \times ha^{-1}$ dose, while the not digested biomass did not show any differences among the N rates.

No toxicity phenomena were detected also at the highest doses of added biomasses and this is an important and positive result in the scope of utilizing these materials in substitution of mineral fertilizer also with high doses of N supply without collateral effect on plant.

For better clarify the effect of the alternative fertilization treatments on lettuce plant parameters in relation of the two soils, radar graphs are reported in Figure 6.



(200 = 200 $kg_N \times ha^{-1}$; 400 = 400 $kg_N \times ha^{-1}$).

Figure 6. Radar graphs of plant parameters related to the fertilization treatments in soil A and B.

Lettuce number of leaves was little affected by soil characteristics, while dry weight, dry matter and total leaf area were evidently influenced by both the soil and the fertilization. On general terms, in the Soil A the percentages of dry matter of lettuce were higher respect to the corresponding values recorded in Soil B; on the contrary, the total leaf areas were lower in Soil A than in Soil B. The water uptake seems to have had a great importance in the two considered systems: probably, it was strongly affected by physical characteristics of the two soils. In Soil A, with about 50% of sand content, the water was less available to plant because of its lower water retention capacity respect to Soil B, so to determine the tendency to reduce the lettuce total leaf area and increase leaves dry matter. On the contrary, in the loamy Soil B, the higher water availability determined a decrease in percentages of lettuce dry matter and the increase of leaf areas, so to give an indication of the dependence of lettuce quality mainly from soil characteristics rather than the fertilization treatments. Anyway, taking into account 200 $kg_N \times ha^{-1}$ of urea as the reference dose for lettuce production, it is relevant that the not-digested solid fraction of livestock manure at 400 $kg_N \times ha^{-1}$ gave the highest value of lettuce dry matter among all the treatments.

For evaluating macro (N, P, K, Mg) and micronutrients (Cu, B, Fe, Mn) use efficiency, biomass dry weight and elemental concentrations were plotted, including curved content isoclines [32],[33]. Each point on the bidimensional plot represent a vectors, taken as control equal to 100% both the concentration and the related dry weight obtained after addition of 200 $kg_N \times ha^{-1}$ urea (intersection point) in graphs (Figure 7 and Figure 8).

Plant tissue composition was significantly affected by the different treatments, depending on the added materials and rate. In relation to both the macro and the micronutrients, it should be remarked that all results were shifted to the limiting vector space (left side of the plot, under 100% lettuce dry matter, corresponding to urea at 200 $kg_N \times ha^{-1}$), representing the reducing growth treatments.

In relation to N (Figure 7), the phytotoxic effect of mineral fertilizer at 400 $kg_N \times ha^{-1}$ is particularly evident in Soil A, where the increase of concentration of N corresponded to the greatest decrease of lettuce dry matter respect to the control; the same severe phytotoxicity was not recovered after treatments with organic biomasses. The most promising results were obtained after addition of not-digested and digested solid fraction of livestock manure at highest dose, giving a dry matter similar to those obtained with the control mineral fertilization, but with a net decrease in N uptake: this finding attests that the N use efficiency was particularly high when 400 $kg_N \times ha^{-1}$ of both organic materials were added to Soil A, since the lack in N prompt availability was not so heavy in limiting plant growth. Such a positive result was not so evident in Soil B, because of the clear reduction of lettuce dry matter (about -50%) after treatments with digested and not-digested materials respect to urea at 200 $kg_N \times ha^{-1}$, even if the 400 $kg_N \times ha^{-1}$ urea application gave a tendency to an excess of N consumption by lettuce, which did not correspond to an increase of dry matter production.

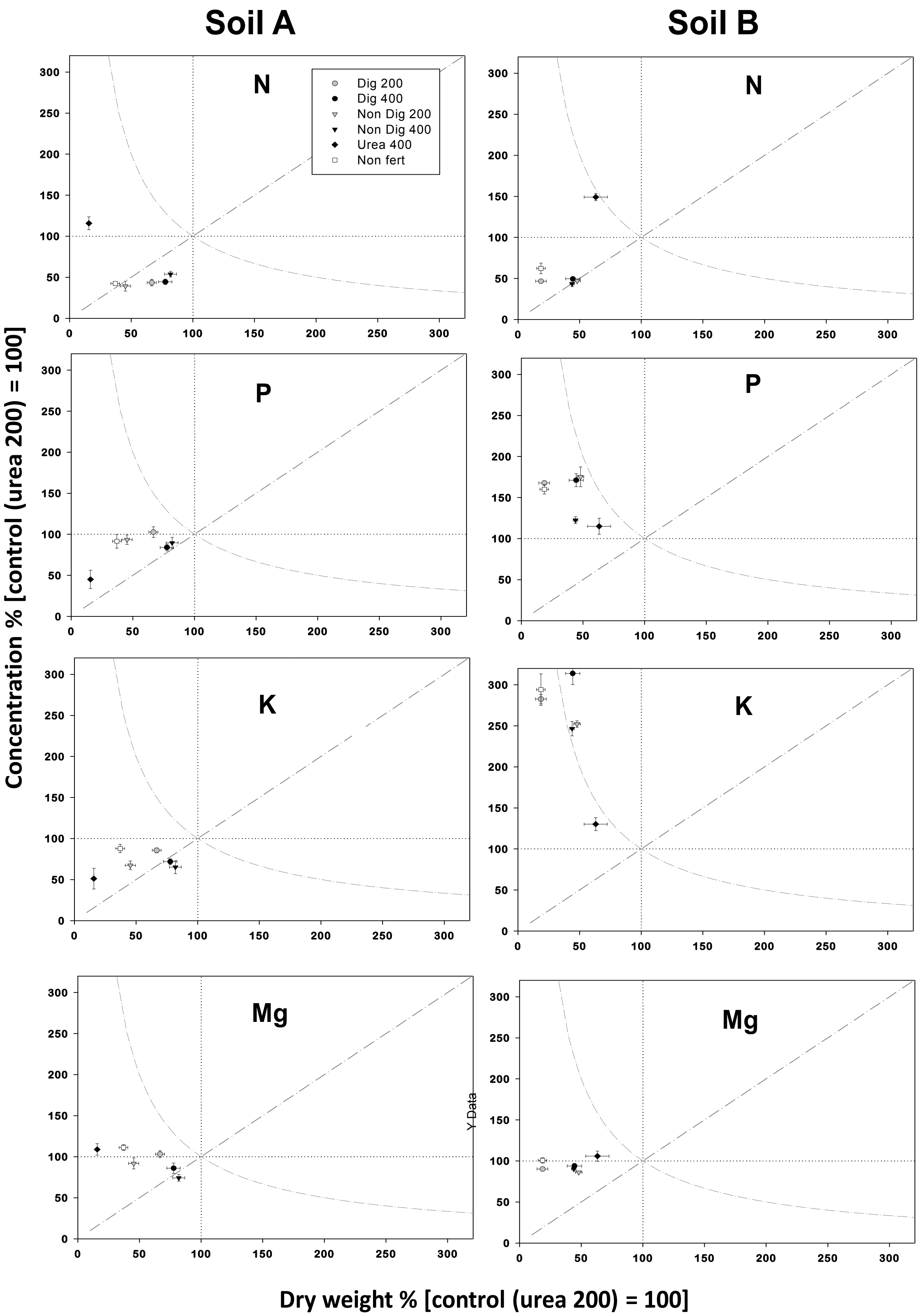


Figure 7. Comparison of relative macronutrients concentration and relative dry weight of aerial part of lettuce plant at the end of growing cycle. Control values of dry weight and concentration used as reference (point of isolines intersection).

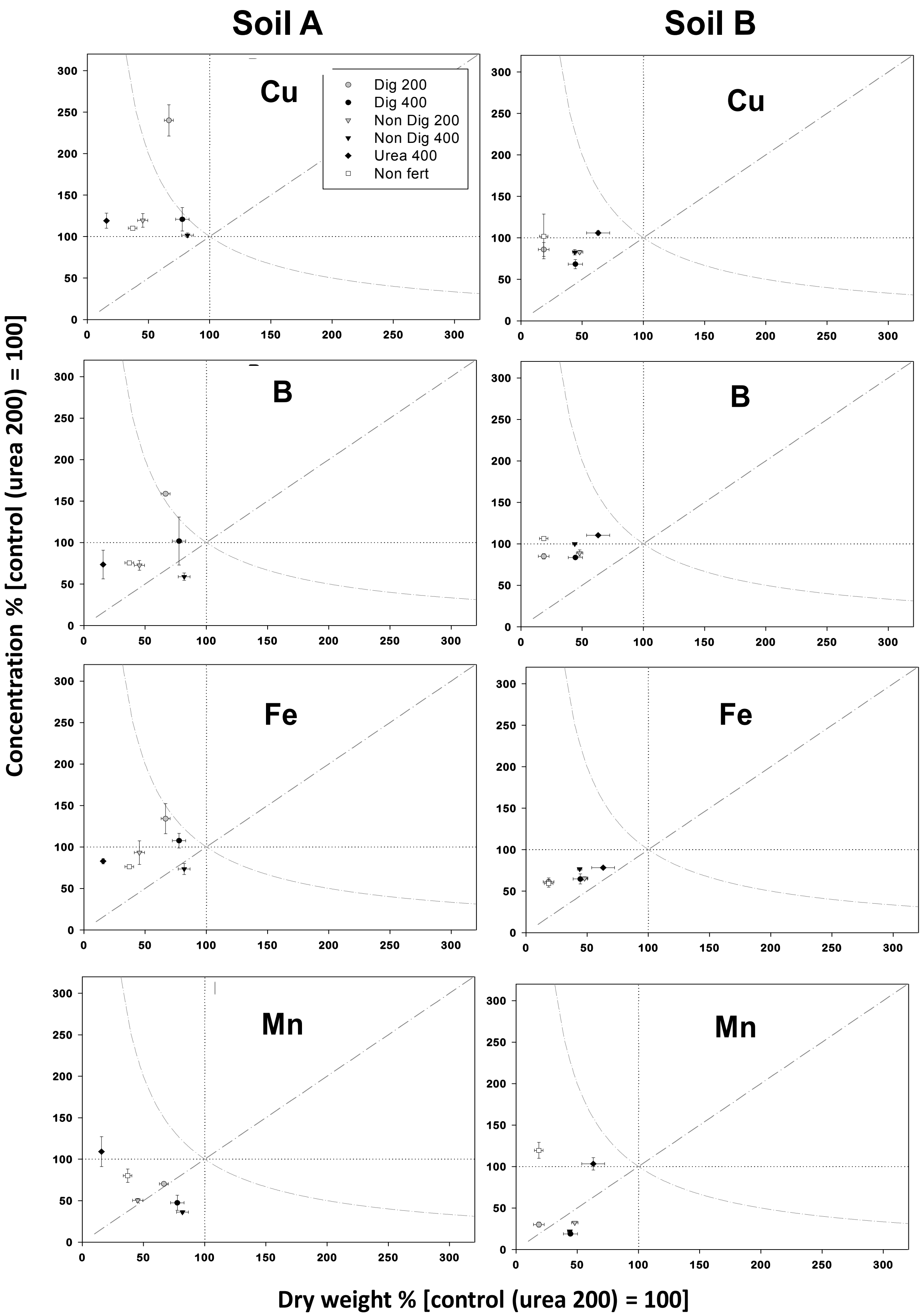


Figure 8. Comparison of relative micronutrients concentration and relative dry weight of aerial part of lettuce plant at the end of growing cycle. Control values of dry weight and concentration used as reference (point of isolines intersection).

Similar results were obtained also for Mg (Figure 7), since again this nutrient concentration seemed to be another limiting factor for lettuce growth, in both the soils.

Different behaviour was recorded for P and K (Figure 7): their related uptakes were strongly affected by both the soils characteristics and the fertilization treatments: while in Soil A the limiting factor for lettuce growth was clearly the soil P and K availability (left space of the graph, under 100% of nutrient concentration for the urea control), in Soil B the effect was opposite (especially for K), since the excess of nutrients appeared to be the main cause of plant growth decrease (left space of the graph, above 100% of nutrient concentration for control 200 $kg_N \times ha^{-1}$), determining a typically defined "nutrient luxury consumption".

In relation to micronutrients Cu, B, Fe and Mn (Figure 8), sometimes their deficiency represented the main limiting factor for lettuce growth (as Fe in Soil B for all the treatments), sometimes the excess of their concentration could have again determined a luxury consumption (as Cu and B in Soil A, after addition of 200 $kg_N \times ha^{-1}$ of digested livestock manure). It is interesting the effect played by soils on Mn lettuce uptake: in Soil A, after addition of 200 $kg_N \times ha^{-1}$ of digested livestock manure, both the Mn uptake and the lettuce dry weight were reduced only of about 30% respect to the values posed to 100% for urea control; on the contrary, in Soil B, the same parameters strongly decreased of about 80%, so confirming the role of soil chemical and physical characteristics on nutrient availability in relation to the different treatments.

3.4. N use efficiency

In Table 3, N use efficiency (calculated as percentage of $N_{uptaken}/N_{fert}$), the residual soil N, the available soil $N\text{-}NO_3$ and $N\text{-}NH_4$ in relation to the different fertilization treatments in soil A and B are reported.

As far as the lettuce N use efficiency is concerned, in both the soils the highest NUE % was obtained after urea application, with the exception of urea at the rate of 400 $kg_N \times ha^{-1}$ in Soil A, which caused an evident phytotoxicity, already manifested by the reduction of lettuce plants growth. In the same soil, higher NUEs were obtained after application of the digested and not-digested solid fraction of livestock manure at the higher 400 $kg_N \times ha^{-1}$ rate. In B soil, while application of the digested biomass at both the doses gave similar NUE values, a quite doubled NUE was obtained after addition of not digested livestock manure at 200 $kg_N \times ha^{-1}$: these positive results could be explained by the presence of higher amount of promptly available N in the not-processed livestock manure respect to the digested one. Anyway, the recorded N use efficiency obtained after the application of both the biomasses was, as expected, lower respect to the urea application at the same rates.

Residual soil N at the end of the cropping cycle after the treatments with digested and not digested biomasses was greater in B soil than in A soil, particularly after digestate application: we suppose that, in this medium-high fertility soil, N-immobilization process took place at higher extent, probably exerted by the soil microflora which was more abundant in B soil, as confirmed by biochemical parameters reported in Table 1. Also the texture could have played a key-role in this process: the textural characteristics of Soil B (~

84%) probably favoured the ammonium fixation, particularly on the loamy-clay components of that soil.

Soil A	NUE ($N_{uptaken}/N_{fert/}$ %)	Residual soil N (g pot^{-1})	Assimilable N-NO_3 (mg pot^{-1})	Exchangeable N-NH_4 (mg pot^{-1})
Not fertilized	0	0,82 a	5,1 c	21,9 b
Urea 200 Kg ha^{-1}	27,1 d	1,49 b	60,2 d	18,3 a
Urea 400 Kg ha^{-1}	2,3 a	1,75 c	146,1 e	25,8 c
Not digested 200 Kg ha^{-1}	4,6 b	1,61 c	4,8 b	26,9 c
Not digested 400 Kg ha^{-1}	6,1 bc	1,89 c	4,1 a	21,9 b
Digested 200 Kg ha^{-1}	4,1 b	1,99 c	4,3 a	23,6 c
Digested 400 Kg ha^{-1}	8,0 c	1,97 c	4,7 b	22,3 bc
Soil B	**NUE ($N_{uptaken}/N_{fert}$ %)**	**Residual soil N (g pot^{-1})**	**Assimilable N-NO_3 (mg pot^{-1})**	**Exchangeable N-NH_4 (mg pot^{-1})**
Not fertilized	0	0,89 a	3,1 a	23,8 ab
Urea 200 Kg ha^{-1}	27,3 d	1,10 b	5,8 c	26,1 b
Urea 400 Kg ha^{-1}	12,8 c	1,23 b	67,1 d	32,5 c
Not digested 200 Kg ha^{-1}	6,8 b	1,43 c	4,2 b	22,5 a
Not digested 400 Kg ha^{-1}	2,4 a	2,44 d	5,8 c	22,1 a
Digested 200 Kg ha^{-1}	2,3 a	2,11 cd	6,5 c	27,4 c
Digested 400 Kg ha^{-1}	2,6 a	2,87 d	6,1 c	25,3 b

Table 3. Nitrogen use efficiency NUE ($N_{uptaken}/N_{fert}$ %), residual soil N (g pot^{-1}), N-NO_3 and N-NH_4 in relation to the different fertilization treatments in soil A and B (average value; different letters means significant differences at P-level<0.05).

In relation to the N forms available in the soils at the end of the experiment, after the treatments with the organic biomasses, the main mineral N fraction is represented by ammonium form, with the exception of urea application, which dramatically promoted the increase of soil nitrate: this finding is extremely positive, since both the digested and the not-digested solid fraction of livestock manure allowed to limit the excess of nitrate ions in the soil solution. Moreover, it is noticeable that the use of these organic biomasses addressed the N immobilization by the microbial biomass activity, guaranteeing an increase of soil residual fertility.

4. Conclusion

Digested, but also the not digested solid fraction of swine livestock manure, showed N availability compatible with those requested from lettuce, so to be used to fertilize short-term horticultural crops. Obtained lettuce plants had a good quality at the end of the experiment, in both the soils and for both the used organic biomasses. Even if the plant dry matter was partially reduced respect to that obtained after mineral fertilization, the macro and micronutrient uptake was lower after organic biomasses addition, indicating an optimization of nutrient use efficiency. Besides, N uptake was well balanced, so to be the N supply able to guarantee a correct development of lettuce.

Since the main aim of our work was to demonstrate the possibility to use these processed biomasses as N source for plant cultivation by limiting the potential nitrate leaching in soil, as normally happened when livestock manure are directly applied to soil, the obtained results confirmed the significant environmental benefits due to the use of anaerobically digested biomasses as organic fertilizers. They showed to be able to promote the development of lettuce crop, probably through the slow release of available N into the soil system, without incurring in toxicity phenomena also when applied at high N supply, being this positive effect particularly effective in a sandy soil, poor in organic matter.

Thus, the biodigestion process represents a great opportunity not only for generating renewable energy, but also to obtain an organic biomass with good fertilizer/amendment characteristics to be applied to the soil, giving the possibility of use alternative nitrogen sources, contemporary promoting plant growth and limiting the environmental impact.

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Acknowledgement

Many thanks are due to Dr. Sergio Piccinini and Dr. Paolo Mantovi of the "Research Centre for Animal Production - Foundation Centre for Studies and Research" (C.R.P.A. – F.C.S.R. S.p.A., Reggio Emilia, Italy) for their kind support in supplying the organic biomasses applied in this work.

Special thanks are due to Prof. Carlo Grignani of the Department of Agronomy, Forestry and Soil Management (Agroselviter) – University of Turin (Italy) and his staff, for providing soils samples for the following research activities.

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