

**USE OF BIOENERGY RESIDUES AS AMENDMENTS: IMPLICATIONS
ON SOIL FERTILITY AND SOIL CARBON SEQUESTRATION**

**UTILISATION DES RÉSIDUS DE PRODUCTION BIOÉNERGETIQUE
COMME AMENDEMENTS: IMPLICATION SUR LA FERTILITÉ
DES SOLS ET LA CAPTURE DE CARBONE**

**UTILIZZO DI RESIDUI DA PROCESSI BIOENERGETICI COME
AMMENDANTI: IMPLICAZIONI SULLA FERTILITÀ DEL SUOLO
ED IL SEQUESTRO DEL CARBONIO**

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Abstract

The increasing use of renewable energy sources as substitutes to fossil fuels has provoked an increase in the production of bioenergy residues. These residues could be effectively used for the recovery and conservation of soil fertility. However, the effect of the organic residues on the soil ecosystem is different depending on their physico-chemical characteristics and, particularly, the knowledge of the impact of bioenergy residues on soil quality is still limited. The aim of this work is to study the effects of different bioenergy residues on C and N mineralization and soil microbial content and activity. A degraded soil (clay 49.7%, pH 7, OC 0.37%) from Southern Spain was amended (0.5% w/w) with four different bioenergy residues (anaerobic digestate, rapeseed meal from biodiesel production, bioethanol residue and biochar) and three other organic residues commonly used as organic amendments (wastewater sludge and two composts). The amended soil was then incubated for 30 days at 20 °C. During incubation soil CO₂ evolution was measured every 4 hours by means of an automatic chromatographic system. After 2, 7 and 30 days of incubation the following parameters were also analysed: K₂SO₄-extractable C, N, NO₃⁻, NH₄⁺ and P, microbial biomass C and some enzymatic activities involved in the cycle of the main nutritive elements (β -glucosidase, arylsulfatase, esterase, alkaline and acid phosphatase and leucine aminopeptidase). Soil addition of the different residues led to a general increase in C and N mineralization, in the availability of nutrients and in the microbial content and activity, but with remarkable different values and dynamics. The only exception was represented by biochar that did not cause any significant variations of the measured parameters with respect to the control. The obtained results demonstrate that bioenergy residues may represent an effective alternative to usual amendments for the recovery and conservation of soil quality. The different physico-chemical

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characteristics of the residues suggest different uses. Rapeseed meal, bioethanol residue and anaerobic digestate are more suited to improve soil biological fertility, while biochar is more appropriated for the enhancement of soil organic matter content and to promote soil C sequestration.

Keywords: *CO₂ emissions; bioenergy residues ; C sequestration; mineralisation; soil microbial biomass*

Résumé

L'augmentation de l'utilisation d'énergies renouvelables comme substituts des combustibles fossiles a provoqué une nette augmentation de la production de résidus bioénergétiques. De tels déchets peuvent être utilisés pour le rétablissement et la conservation de la fertilité des sols. Pourtant, l'effet des résidus organiques sur l'écosystème du sol est différent selon ses caractéristiques physico-chimiques. En particulier, les connaissances concernant l'impact des résidus bioénergétiques sur la qualité du sol sont encore limitées. Nous avons étudié les effets de résidus de différents processus bioénergétiques sur la minéralisation de C et N ainsi que sur le contenu et l'activité des microorganismes. Un sol dégradé (argile 49.7 %, pH 7, CO 0.37 %) provenant de l'Espagne méridionale a été amendé (0.5 % m/m) avec quatre différents résidus bioénergétiques (déchets de la digestion anaérobie, farine de colza de la production de biodiesel, résidus de la production de bioéthanol et de charbon de bois) et trois résidus organiques habituellement utilisés comme amendements (boue de dépurateur et deux types de compost). Le sol, après amendement, a été incubé 30 jours à 20 °C. Au cours de l'incubation, l'évolution de CO₂ a été mesurée à intervalles de quatre heures par chromatographie. Après 2, 7 et 30 jours les paramètres suivant ont été analysés: C, N, NO₃⁻, NH₄⁺ et P extractible par K₂SO₄, C de la biomasse microbienne et certaines activités enzymatiques impliquées dans le cycle des principaux éléments nutritifs (β-glucosidase, arylsulfatase, estérase, phosphatase alcaline et acide ainsi que leucyl-aminopeptidase). L'addition de divers résidus au sol a provoqué en générale une augmentation de la minéralisation de C et N, de la disponibilité des nutriments et du contenu et activité des microorganismes ; tout ceci avec de significatives différences aussi bien en terme de quantité que de dynamique. L'addition de résidus de la production de charbon de bois n'a pas provoqué de variation significative des paramètres mesurés par rapport au témoin. Les résultats obtenus démontrent que les résidus bioénergétiques peuvent représenter une alternative efficace aux amendements habituels pour le rétablissement et la conservation de la qualité du sol. Les différentes caractéristiques physico-chimiques des résidus suggèrent diverses modalités d'utilisation. La farine de colza, le résidu de la production de bioéthanol et les produits de la digestion anaérobie sont plus indiqués pour améliorer la fertilité biologique du sol alors que le charbon de bois est plus approprié pour augmenter le contenu en matière organique et favoriser la capture de C.

Mots clés: *émissions de CO₂; résidus bioénergétiques; stockage de C; minéralisation; biomasse microbienne*

Riassunto

L'aumento dell'utilizzo di energie rinnovabili come sostituti dei combustibili fossili ha provocato un consistente incremento nella produzione di residui bioenergetici. Tali residui possono essere convenientemente utilizzati per il recupero ed il mantenimento della fertilità dei suoli. Tuttavia, l'effetto dei residui organici sull'ecosistema del suolo è diverso a seconda delle sue caratteristiche fisico-chimiche. In particolare le conoscenze sull'impatto dei residui dei processi bioenergetici sulla qualità del suolo sono ancora limitate. Lo scopo di questo lavoro era pertanto lo studio degli effetti di residui da diversi processi bioenergetici sulla mineralizzazione di C e N ed il contenuto ed attività dei microorganismi del suolo.

Un suolo degradato (argilla 49.7%, pH 7, CO₂ 0.37%) proveniente dalla Spagna meridionale è stato ammendato (0.5% p/p) con quattro diversi residui da processi bioenergetici (residuo della digestione anaerobica, farina di colza dalla produzione di biodiesel, residuo della produzione di bioetanolo e biocarbone) e tre residui organici comunemente usati come ammendanti (fango di depurazione e due tipi di compost). Il suolo ammendato è stato poi incubato per 30 giorni a 20 °C. Durante l'incubazione è stata misurata l'evoluzione della CO₂ ogni 4 ore mediante un sistema cromatografico automatizzato. Dopo 2, 7 e 30 giorni di incubazione sono stati analizzati i seguenti parametri: C, N, NO₃⁻, NH₄⁺ e P estraibili con K₂SO₄, C della biomassa microbica e alcune attività enzimatiche implicate nel ciclo dei principali elementi nutritivi (β -glucosidasi, arilsulfatasi, esterasi, fosfatasi acida e alcalina e leucina aminopeptidasi).

L'aggiunta dei diversi residui al suolo ha provocato in generale un aumento nella mineralizzazione di C e N, nella disponibilità di nutrienti e nel contenuto ed attività dei microorganismi, ma con significative differenze nella quantità e dinamica. L'unica eccezione è stata costituita dal biocarbone che non ha causato variazioni significative dei parametri misurati rispetto al controllo. I risultati ottenuti dimostrano che i residui bioenergetici possono rappresentare un'alternativa efficace ai comuni ammendanti per il recupero ed il mantenimento della qualità del suolo. Tuttavia le diverse caratteristiche chimico-fisiche dei residui suggeriscono differenti modalità di utilizzo. La farina di colza, il residuo della produzione di bioetanolo ed il digestato sono più indicati per migliorare la fertilità biologica del suolo, mentre il biocarbone è più appropriato per aumentare il contenuto di materia organica del suolo e favorire il sequestro del C.

Parole chiave: emissioni di CO₂; residui bioenergetici; sequestro di C; mineralizzazione; biomassa microbica

Introduction

In the last decades, modern agriculture practices have led to a widespread decrease in soil organic matter (SOM), particularly in temperate regions. The decline in SOM is accompanied by a reduction in soil fertility and contributes to the onset of several degradative processes (such as erosion, compaction, salinisation, nutrient deficiency, loss of biodiversity, desertification). One of the most effective

strategies to offset SOM decline is the amendment of soil with organic residues (Lal *et al.*, 1999).

The increasing use of renewable energy sources as substitutes to fossil fuels has provoked an increase in the production of bioenergy residues. These residues are rich in organic matter and nutritive elements and therefore could be effectively used as soil amendments for the recovery and conservation of soil fertility. However, due to the recent availability of these residues, very limited research has been done to determine how their agronomical utilization would influence soil quality. Organic residues present great variations in their physico-chemical properties and therefore they affect the soil ecosystem in different ways. Hence, an evaluation of the impact of residues on soil quality is essential to fully exploit their potential as amendments and to avoid adverse environmental effects.

The aim of this work was to evaluate the effects of different bioenergy residues on C and N mineralization and soil microbial size and activity and compare them to other more commonly used organic amendments.

Materials and methods

The experiments were carried out with a degraded soil from Llano de la Perdiz, Granada (Southern Spain). The main characteristics of the soil are shown on Table 1.

Soil type (FAO/UNESCO 2006)	Chromic luvisol	Table 1
Sand (%)	32	<i>Soil characteristics</i>
Silt (%)	17	
Clay (%)	51	
CEC (meq _e /100g)	6.78	
TN (%)	0.11	
TC (%)	0.92	
CaCO ₃ (%)	0.05	
pH	7.0	
OC (%)	0.37	
EC (μS cm ⁻¹)	41	

Four bioenergy residues and 3 organic residues commonly used as organic amendments were selected for incubation experiments. The bioenergy residues were: pig slurry digestate (37.9% C, 4.4% N), rapeseed meal from biodiesel production (45.9% C, 6.0% N), residue obtained after bioethanol production from wheat starch (48.5% C, 6.2% N), and green waste biochar produced by continuous slow pyrolysis at 550 °C (86.3% C, 0.3% N). The organic residues consisted of compost from vine shoots (34.5% C, 1.5% N), household waste compost (34.4% C, 2.3% N) and sewage sludge from a wastewater treatment plant (38.4% C, 4.8% N). The soil was pre-conditioned at 40% of water holding capacity and 20 °C under aerobic conditions for 5 days, subsequently amended with the residues at a rate of 0.5% (w/w) and incubated for 30 d at 20 °C. The rate of application was calculated

to reproduce the soil addition of the residues at the rate of 20 ton ha⁻¹, representing a typical agronomic rate for amendments. During incubation, soil CO₂ evolution was continuously measured every 4 hours, while aliquots of soils were taken after 2, 7 and 30 days of incubation for analysis of K₂SO₄ extractable NH₄⁺, NO₃⁻, organic C (EOC) and N (EN) and enzymatic activities (Arylsulphatase, β-glucosidase, alkaline phosphatase, acid phosphatase and leucine aminopeptidase). Soil microbial biomass was also analysed after 2 and 30 days of incubation.

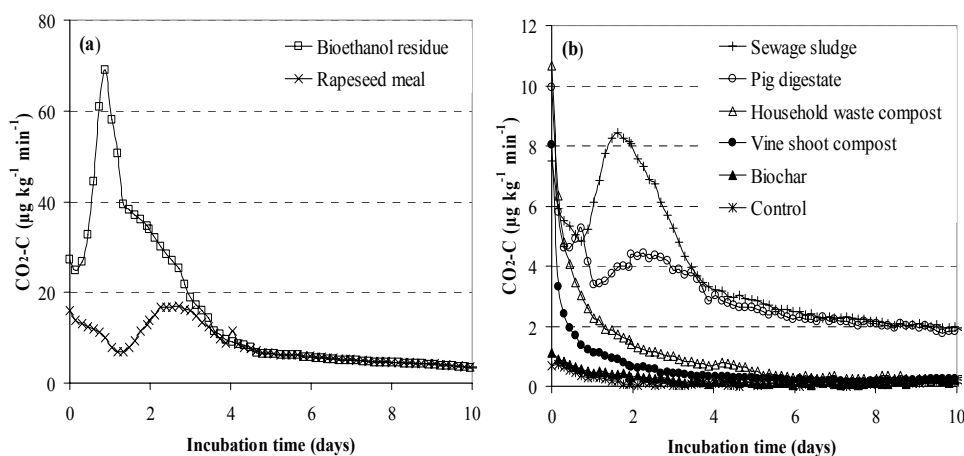
CO₂ evolution was determined by means of an automated system for continuous gas sampling and analysis (Mondini *et al.*, 2010). Extractable NH₄⁺, NO₃⁻, organic C and N were determined in a 1:4 (w/v) K₂SO₄ 0.5M extract. Extractable NH₄⁺ was determined by a modified colorimetric method based on Berthelot's reaction (Sommer *et al.*, 1992). The content of NO₃⁻ was measured by reading the absorbance at 220 nm and subtracting the absorbance at 275 nm caused by organic matter. Extractable organic C and EN were measured using a TOC-VCSN analyser (Shimadzu). Soil microbial biomass C (B_C) was determined by the fumigation-extraction method (Vance *et al.*, 1987). The different enzymatic activities (Arylsulphatase, β-glucosidase, leucine aminopeptidase and alkaline and acid phosphatase) were measured in soil extracts utilizing a fluorescence method.

Results and discussion

C and N mineralization. Figure 1 shows CO₂ emissions during the first 10 days of incubation.

As it can be seen, dynamics and amount of soil CO₂ evolution were greatly affected by the different residues. The highest CO₂ emissions were recorded with bioethanol residue followed by rapeseed meal (Figure 1a).

Figure 1 - CO₂ emissions during the first 10 days of incubation of amended soil. Note the different scale of the y axes in the two graphs.



These two residues had a similar C and N content, with a C/N ratio around 7.8. On the other side, the respiration rate in biochar amended soil was not significantly different from the control (Figure 1b). This residue presented a very high C/N ratio, with a very low N content.

Cumulative extra CO₂-C (difference in cumulative respiration between amended and control soil) ranged from 0 to 10.9 % of the C added with the residues (Table 1).

Table 1 - Balance of C and N mineralization in amended soil after 30 d of incubation. Extra CO₂-C is the difference in cumulative respiration between amended and control soil. Net mineral N is the difference between NO₃⁻ plus NH₄⁺ in the amended soil and NO₃⁻ plus NH₄⁺ in the control soil. Negative values indicate net N immobilization.

Residue	Extra CO ₂ -C		Net mineral N	
	(μg g ⁻¹)	(% of added C)	(μg g ⁻¹)	(% of added N)
Vine shoot compost	15.1	0.9	-3.6	-4.8
Household waste c.	19.1	1.1	-6.0	-5.2
Biochar	1.6	0.0	-0.4	-2.9
Pig digestate	63.7	3.4	40.8	18.5
Rapeseed meal	158.2	6.9	123.8	41.3
Bioethanol residue	263.2	10.9	95.4	30.8
Sewage sludge	73.2	3.8	46.0	19.2

Soil amendment with bioenergy residues caused, with the exception of biochar, a remarkable increase in the content of extractable N indicating an increase in soil N availability (Figure 2).

Net (control subtracted) mineral N (NO₃⁻ + NH₄⁺) at the end of the experiment showed that the two composts led to a slight N immobilization, biochar did not cause any mineralisation, while the remaining residues caused an increase in net mineral N ranging from 18.5 to 41.3% of added N (Table 1).

This corresponds to an increase in available N in the range 163-495 kg N ha⁻¹.

The mineral N was almost entirely composed of NO₃⁻ (Figure 3) and the highest values were recorded for bioethanol residue and rapeseed meal.

The amount of mineral N at the end of the incubation was significantly related to the EOC/EN ratio (data not shown), suggesting that the amounts of readily available C and N are better indicators of the rate of N mineralisation than the total C and N content of the residue.

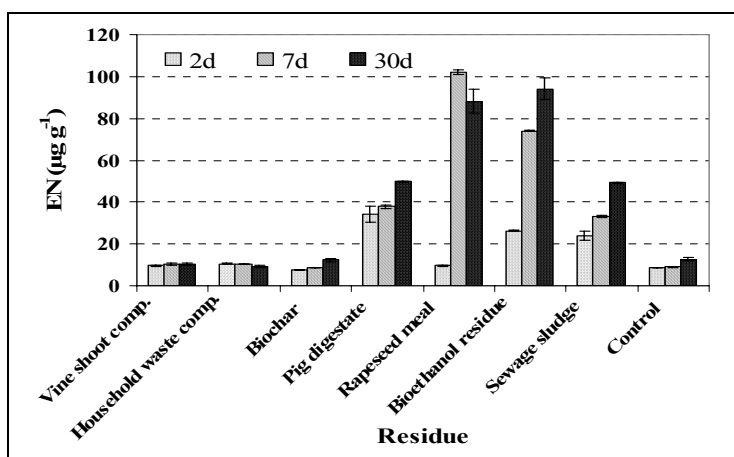


Figure 2

EN content in amended and control soil after 2, 7 and 30 days of incubation.

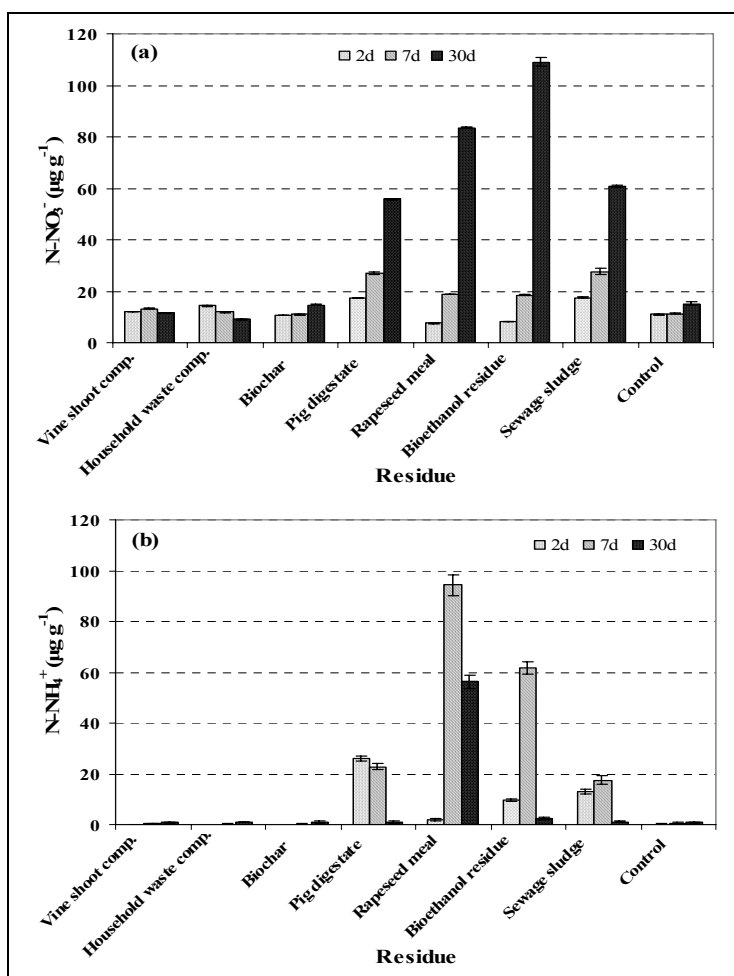


Figure 3

NO₃⁻ and NH₄⁺ content in amended and control soil after 2, 7 and 30 days of incubation.

Soil microbial biomass and enzymatic activity. Soil amendment with organic residues caused, with the exception of biochar, a general increase in the size and the hydrolytic activity of soil microbial biomass, although with highly significant differences among the residues.

The highest increase in soil microbial biomass C content was obtained in the soil amended with bioethanol residue followed by rapeseed meal (Figure 4). In the case of bioethanol residue, such increase was not sustained during the whole incubation period and there was a sharp decrease of the microbial biomass content at the end of the incubation to values that were, however, significantly higher than the control. Such a decrease was likely to be due to the exhaustion of the readily available C and N (data not shown). The other residues caused a less relevant increase in the size of soil microbial biomass after 2 days of incubation, but this increase was maintained throughout the whole incubation period.

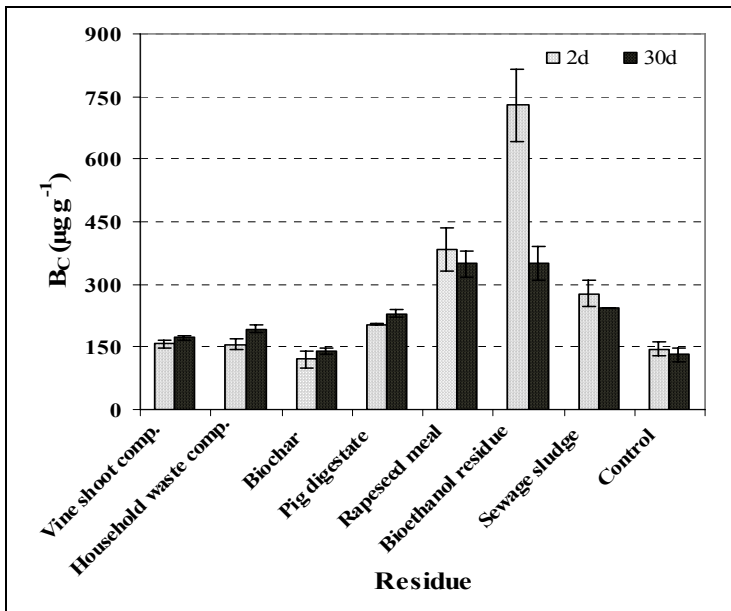


Figure 4

Microbial biomass C after 2 and 30 days of incubation.

Figure 5 reports the values of β -glucosidase and leucine aminopeptidase activities in amended soil as an example of the typical response of the measured enzymatic activities to soil amendment. As for microbial biomass, higher values of β -glucosidase activity were recorded for soil amended with bioethanol residue followed by rapeseed meal (Figure 5a), which were characterized by a high amount of readily available C, measured as water soluble organic C (data not shown). In the case of leucine aminopeptidase activity, higher values were recorded after 7 days of incubation for bioethanol residue, rapeseed meal, pig digestate and sewage sludge (Figure 5b). This reflected the high content of total N in these residues (N > 4.4%).

The increase in hydrolytic enzymatic activity is an indication of an enhanced capacity of the soil to carry out important ecosystem functions such as the degradation of soil organic matter and the cycling of nutritive elements.

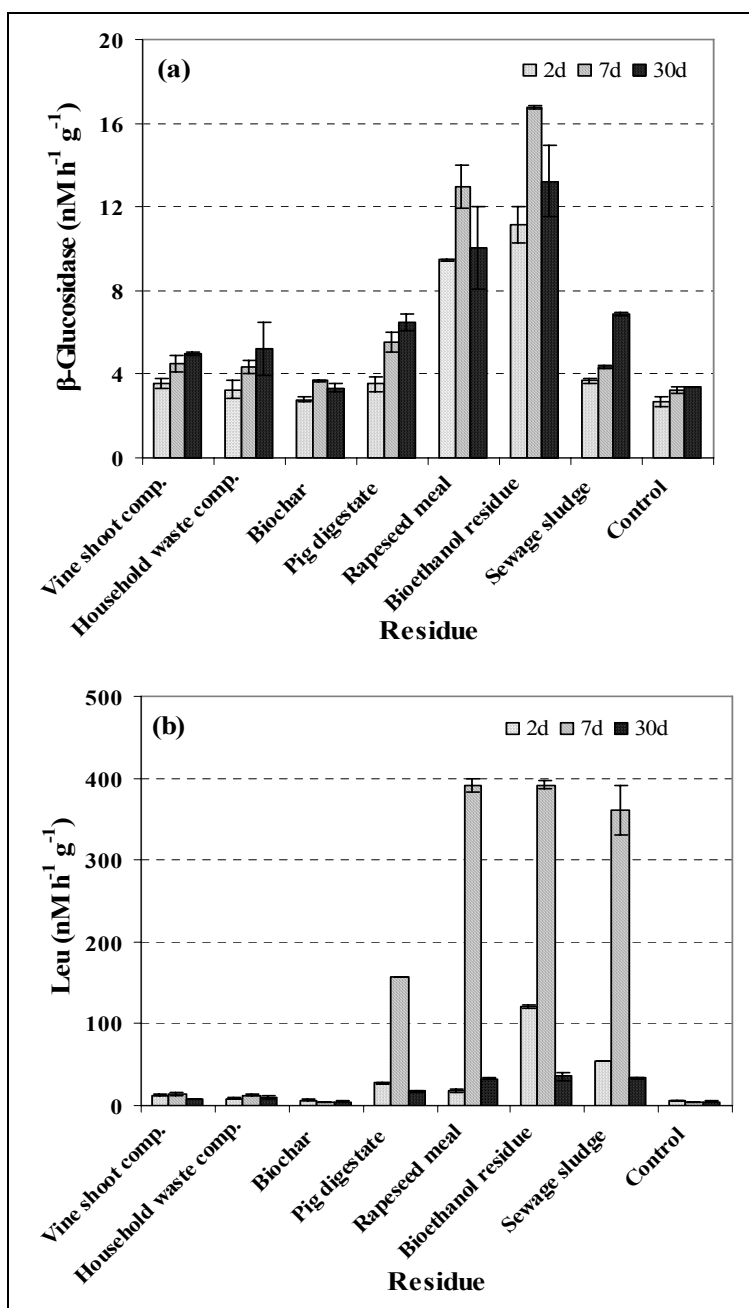


Figure 5

β -glucosidase and leucine aminopeptidase activities after 2, 7 and 30 days of incubation.

As a whole and according to their impact on biochemical soil properties, the residues can be ranked as follows: bioethanol residue, rapeseed meal > anaerobic digestate, wastewater sludge > composts > biochar.

Conclusions

The results obtained demonstrate that bioenergy residues may represent an effective alternative to usual amendments for the recovery and conservation of soil quality.

The different physico-chemical characteristics of the residues suggest different uses.

Rapeseed meal, bioethanol residue and anaerobic digestate are best suited to improve soil biological fertility. They can provide significant amounts of available N for plants, along with a source of readily available C for microorganisms growth and activity.

Biochar is more appropriated for the enhancement of soil organic matter content and to promote soil C sequestration, due to the very low rate of soil mineralization. The increase of soil organic matter usually requires high loads of exogenous organic matter, but this is favoured in the case of biochar by the particular biological inertia of this residues resulting in a limited alteration of soil equilibrium.

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