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HIGH RESOLUTION NMR TO EVALUATE THE INFLUENCE EXERTED BY GREEN COMPOST, VERMICOMPOST AND SOLID ANAEROBIC DIGESTATE ON THE PRIMARY METABOLOME OF LETTUCE

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Introduction

The agricultural field deals with serious environmental challenges, including the increasing degradation of fertile soils and the decrease of organic matter content. These conditions are very widespread and, other than compromising plant food production, involve approaches based on the consumption of non-renewable mineral resources and the use of products that, in the long term, may be detrimental to the soil. Therefore, the principles of the circular economy call for the recovery and valorisation of waste biomass to develop materials to be applied in soil and useful to restore its quality. The goal is to obtain products that can improve soil fertility and resilience, while optimizing the production and quality of primary production. The present study is part of the research activities for the task 8.3.2 of the PNRR AGRITECH and aims to investigate the effects implied by the use of different products (solid digestate, vermicompost and compost) as sustainable organic fertilizers deriving from biological and chemical valorisation of waste biomasses which are relevant for the Campania region. These products were tested on model plants (*Lactuca sativa* species) in order to exclude phytotoxicity, verify biostimulation effects and identify ideal doses. In particular, we evaluated the impact exerted on the nitrogen content and primary metabolome of lettuce leaves resulting from the application of the studied products (provided at the optimal doses), comparing the responses with samples obtained either without any addition to the growth substrate (CONTROL) or treated with mineral fertilizer (MIN).

Materials and Methods

Soil fertilizers: Based on a collaboration with the C&F ENERGY Biogas Plant, **SOLID DIGESTATE** resulted from the anaerobic digestion of buffalo livestock manure and was enriched in ammonia nitrogen collected through the stripping of the digestate liquid phase. Part of the solid digestate was then reprocessed and converted to **VERMICOMPOST** via an earthworm-based bioreactor. Based on a collaboration with the OP TERRAMORE, a **GREEN COMPOST** deriving from the valorisation of waste from the production of IV gamma vegetables (red chicory, spinach and escarole) was produced. These products were tested on lettuce via greenhouse trials and using peat as substrate. Agronomic tests permitted to define the optimal doses which corresponded to 10, 20 and 40 %(V:V) for compost, vermicompost and digestate, respectively.

Protein nitrogen content: Kjeldahl method involving acid digestion of dried biomass, followed by distillation and titration (3 replicates per type).

^{1}H NMR metabolomics: ^{1}H 1D NMR spectra were acquired at 25 °C on aqueous extract (pD 6 with TMSPA) by using a 400 MHz Bruker magnet, equipped with Broad-Band Inverse (BBI) probe and applying a solvent suppression (at least 7 replicates per type). Spectral data were processed by several multivariate statistical analyses.

Results

Protein nitrogen content: **Table 1** shows the contents (%) in nitrogen and crude protein detected in lettuce leaves and resulting from the investigated treatments. Nitrogen content ranged within 1.5 and 4%. As expected, the highest values were found in samples MIN. The samples with the lowest nitrogen content were the controls without fertilizer, while those treated with studied soil improvers led to intermediate values. This response suggests that the used products, in addition to stimulating root activity and promoting higher production, provide a slow-release source of nitrogen.

Table 1. Nitrogen and crude protein contents (%) in dried lettuce leaves (Conversion factor of 6.25)

	% N	% Crude protein
Control (1)	1.585 ± 0.267	9.903 ± 1.672
Mineral fertilizer (1)	3.789 ± 0.402	23.681 ± 2.513
Compost	1.970 ± 0.127	12.314 ± 0.794
Control (2)	1.506 ± 0.069	9.411 ± 0.433
Mineral fertilizer (2)	4.048 ± 0.099	25.300 ± 0.619
Vermicompost	2.738 ± 0.030	17.115 ± 0.186
Solid digestate	2.836 ± 0.030	17.728 ± 0.186

NMR-based Metabolomics: A semiquantitative comparison of 1D NMR proton spectra of aqueous extracts of lettuce leaves was conducted using several multivariate statistical techniques, including PCA (**Figures 1-4**). The PCA score-plots proved that each of the studied treatments induced the development of diagnostic and specific compositional traits, attributable to different and treatment-dependent contents of amino acids, organic acids, carbohydrates and phenolic compounds. In all cases, it was assessed a net difference among CONTROL, MIN and ORGANIC FERTILIZER composition. In particular, the control samples, which exhibited the lowest nitrogen content, appeared isolated from the other samples. MIN samples, which exhibited the highest nitrogen content, showed a metabolomic profile similar to those treated with the three soil improvers. Interestingly, the metabolome of samples treated with the experimental soil improvers determined the placement of the scores in an intermediate PCA position, included within control and MIN barycenters. These preliminary results highlight the impact of the studied products on qualitative and compositional aspects of lettuce, with responses approaching to those resulting from mineral fertilization.

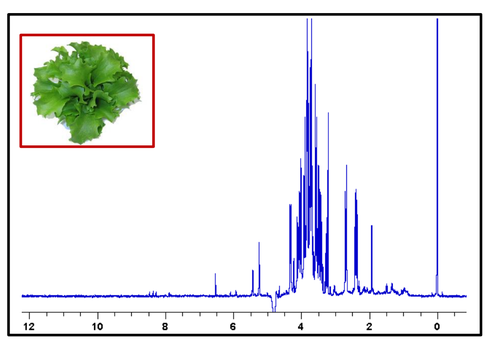

Figure 1. Representative ^{1}H 1D NMR spectrum of lettuce leaves extract (pD6, added with TMSPA as internal standard).

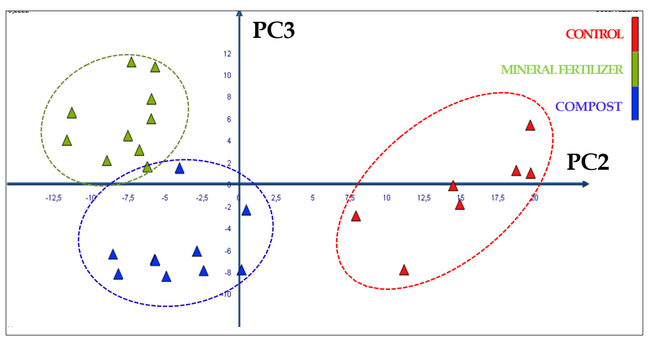

Figure 2. PCA score-plot based on ^{1}H NMR spectra of Control and samples treated with either Mineral fertilizer or Compost (10% V:V). PC2 vs PC3, total explained variance of 29.02%.

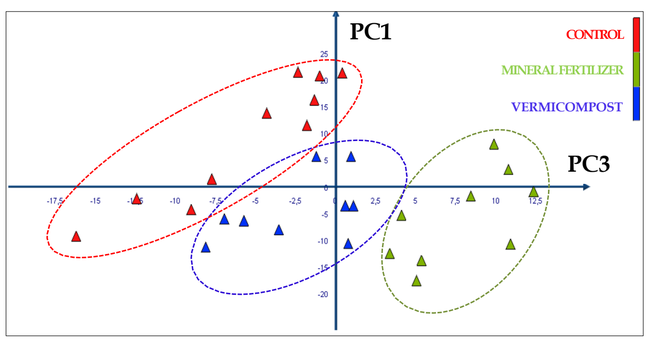

Figure 3. PCA score-plot based on ^{1}H NMR spectra of Control and samples treated with either Mineral fertilizer or Vermicompost (20% V:V). PC1 vs PC3 35.21% Total explained variance.

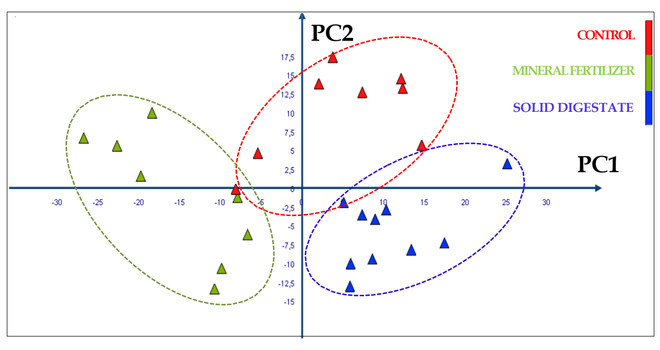

Figure 4. PCA score-plot based on ^{1}H NMR spectra of Control and samples treated with either Mineral fertilizer or Solid Digestate (40% V:V). PC1 vs PC2 50.27% Total explained variance.

Conclusions

The results confirm that all investigated products, which result from a virtuous process of waste biomass valorization, can act as effective plant biostimulants and soil conditioners when administered in appropriate doses. They can offset mineral fertilizers in the cultivation of plants developing fourth range products, impacting positively on production, quality and compositional aspects. These results candidate the investigated products as viable circular economy strategies, serving as an alternative solution to promote the sustainability and resilience of agricultural systems, and increase the organic matter in soil.