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Short- and long-term effects of a single application of two organic amendments

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Abstract. A frequent side-effect of soil treatment with organic amendments is the slow release of harmful metals deriving from the initial matrices, mainly municipal waste and manure from intensive farming. Contamination is amplified by repeated treatments, which is a common practice to maintain soil fertility. The aim of the present research was to compare, in a mesocosm trial, short (one year)- and long-term (ten years) effects of a single application of compost or a mixture of compost and poultry manure to limestone waste. Attention was focused on pH, organic matter content, metal availability, and microbial biomass and activity. Amendment ecotoxicity at ten years after application was also evaluated. A single application reduced the metal availability and metabolic quotient (an index of stress condition). In the long term, an overall improvement of the environmental conditions has been observed, as the microbial biomass increased, respiration decreased (suggesting low energy requirement) and mineralization activity decreased (likely due to high recalcitrance of residual organic matter). In the brief term, poultry manure played a significant role in improving the environmental conditions as it contributed to reduce the metal availability and to enhance the microbial biomass and activity. In the long term, the overall conditions of both the organic amendments appeared favorable for organisms as low ecotoxicity occurred.

Key words: compost; ecotoxicity; heavy metals; microbial activity; microbial biomass; poultry manure.

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INTRODUCTION

Organic amendments (i.e., compost, poultry manure, peat, sewage sludge, or others) increase soil organic matter and nutrient concentrations and enhance soil structure, porosity, and water penetration; hence, they are widely employed to improve poor or degraded soils (Gigliotti et al. 1996, Celik et al. 2004, Bastida et al. 2007, Businelli et al. 2009, Hernandez et al. 2015). Amendments rich in humic substances such as quality compost release nutrients gradually (Schnitzer and Khan 1978, Cooperband et al. 2002), and hence, they have a protracted effect on plants and edaphic microorganisms (Caravaca et al. 2002); in contrast, amendments rich in nitrogen (i.e., manure) induce rapid plant growth (Hesse et al. 2004, Delgado et al. 2012, da Silva Oliveira et al. 2017). By releasing organic exudates through the roots, plants can increase the biomass and activity of edaphic microorganisms (Singh et al. 2004, Chaparro et al. 2014).

A possible side-effect of soil treatment with organic amendments is the release of pollutants. Bioaccumulation of inorganic (mainly harmful metals) and persistent organic pollutants (e.g., PCBs and dioxins) is an unavoidable consequence of compost production and is becoming a major risk due to increasing use of municipal solid waste, sewage sludge, or manure from industrial plants as starting materials (Achiba et al. 2009). The applications of organic amendments are often repeated several times in order to improve soil properties and obtain high yields, but this may increase contamination (Iwegbue et al. 2007, Kidd et al. 2007). Long-term effects of repeated applications of organic amendments have been investigated quite extensively (Ros et al. 2006, Achiba et al. 2009, Hernandez et al. 2015), but the effects of single applications are still unknown.

In Campania Region (southern Italy), concrete industry produces about 14,026,000 t/yr of limestone debris whose disposal is a major environmental problem (BURC 2006). In parallel, ~2,257,500 t of municipal waste was collected in the last year, with 60% being used for compost production (ORR 2016). With the objective of evaluating the application of organic amendments for reducing the impact of limestone waste while avoiding contaminant accumulation, we investigated short- and long-term effects (one and ten years, respectively) of a single application of two types of organic amendments to limestone material from a local quarry.

MATERIALS AND METHODS

Mesocosm setting up

The research was carried out in mesocosm trials. In March 2004, 15 pots 1 m in diameter were filled to about 60% of total height (60 cm) with limestone debris with 1–4 cm granulometry, from a quarry in the Caserta area (Campania, Italy). One mesocosm containing compost only was kept as control (C); in seven mesocosms (C_p), compost mixed with expanded clay pellets (70:30 = v:v) was placed above the limestone debris; and in the other seven mesocosms (CP_p), a mixture of compost and poultry manure plus wheat husk and expanded clay pellets (60:10:30 = v:v:v) was placed on the limestone debris (Fig. 1). The amount of compost added to each pot was 150 kg f.w., equivalent to about 1900 t/ha. The compost used for the experiments was produced by a local company (Pomigliano Ambiente S.p.A., Pomigliano, Naples, Italy) from the organic share of municipal wastes and green refuses from tree pruning. Two 1-yr-old specimens of native sclerophyllous shrubs, *Laurus nobilis* L. (bay tree), *Phillyrea angustifolia* L. (jasmine box), and *Quercus ilex* L. (holm oak), were transplanted in C_p and CP_p mesocosms, to a total of six specimens per pot. The mesocosms were put outdoors in the Botanical Garden of Naples and adequately irrigated with distilled water.

From each mesocosm and at each sampling time, that is, March 2004, March 2005, and March 2014, three samples of substrate were collected from the surface layer (0–10 cm) and mixed into a representative composite sample (Fig. 1). The samples were sieved (<2 mm) and analyzed for physico-chemical and biological parameters; in addition, ecotoxicological assays were performed only for the samples collected in 2014.

Physico-chemical and biological analyses

An aliquot of each composite sample was oven-dried (75°C, until constant weight) for physico-chemical analyses. pH in water (1.0:2.5 = w:w) was measured using a pH meter. The organic matter content and total C, N, and metal (Cd, Cr, Cu, Ni, and Pb) concentrations were measured on samples powdered by an agate mortar and pestle (Fritsch Analysette Spartan 3 Pulverisette 0), whereas the metal available fractions were determined in non-pulverized samples. The metals to determine were selected among the contaminants indicated in the National Law (Legislative Decree 75/2010) for organic amendments for agricultural use.

In order to characterize the substrates, the total C, N, and metal concentrations were measured only at the beginning of the experiment. Instead, the organic carbon was measured on samples collected at each sampling time in order to calculate the organic matter content. The organic carbon, in samples previously treated with HCl (10%), and the total concentrations of C and N were evaluated by gas chromatography (CNS Analyzer; Thermo Finnigan, Milan, Italy). The organic matter content was obtained multiplying the organic carbon for 1.724 (Pribyl 2010).

In order to obtain the total concentrations of Cd, Cr, Cu, Ni, and Pb, the substrate samples were previously digested by HF (50%) and HNO_3 (65%) in the 1:2 (v:v) ratio in a microwave oven (Digestion/Drying Module mls 1200; Milestone, Leutkirch, Germany); to obtain the available metal fractions, the substrate samples were extracted with diethylenetriamine pentaacetic acid, $CaCl_2$, and triethanolamine at pH 7.3 ± 0.05

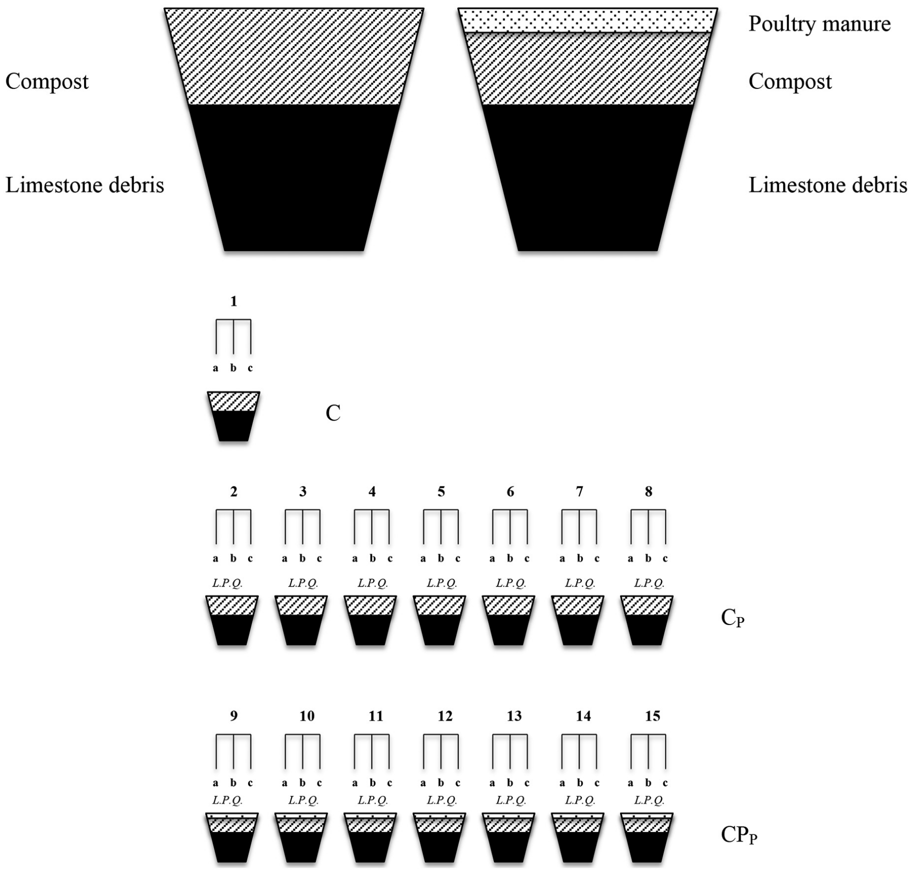

Fig. 1. Scheme of mesocosm setting up (top) and of the sampling procedure (bottom). C, mesocosm with compost without plants; C_p, mesocosms with compost and plants; CP_p, mesocosms with the mixture of compost and poultry manure and plants; *L*, *Laurus nobilis* L. (bay tree); *P*, *Phillyrea angustifolia* L. (jasmine box); *Q*, *Quercus ilex* L. (holm oak); a–c, points of sampling for each mesocosm at each sampling time; 1–15, homogeneous samples from each mesocosm.

(Lindsay and Norvell 1978). The metal concentrations were measured by atomic absorption spectrometry, via graphite furnace (SpectrAA 220 FS; Varian, Sidney, Australia).

Microbial and fungal biomasses and biological activity were measured within three days from collection in samples stored at 4°C. Microbial carbon (C_{mic}) was evaluated by the method of substrate-induced respiration according to Anderson and Domsch (1978), while microbial activity was estimated as potential respiration. Microbial C was measured by CO_2 evolution from soil (3 g of each soil sample) in response to the addition of glucose (2 mL of 75 mmol/L D-glucose), an easily mineralizable substrate, after incubation for 5 d in the dark at 25°C and 55% water holding capacity. CO_2 values were corrected for the CO_2 measured in a blanc and were reported as mg of microbial carbon per g soil. The potential respiration of soil samples was estimated as CO_2 evolution in standard conditions (10 d of incubation at 25°C in the dark, at 55% water holding capacity) and was expressed in mg CO_2 evolved per g soil per time unit.

CO_2 evolution was measured after incubation by NaOH absorption followed by two-phase titration with HCl (Froment 1972). The metabolic quotient, qCO_2 (mg C–CO_2/mg C_{mic}), that is, the degree of activity of the microbial biomass, and the coefficient of endogenous mineralization (CEM, mg C–CO_2/g C_{org}), that is, the rate of organic C mineralization, were calculated using respiration data and microbial C and organic C data, respectively.

Total fungal biomass was assayed by membrane filter technique (Sundman and Sivelä 1978), after staining with aniline blue, determining hypha length by the intersection method (Olson 1950) with an optical microscope (Optika, B-252). Soil samples were suspended in a solution (1 g of fresh soil in 100 mL) of phosphate buffer (60 mmol/L, pH 7.5) and homogenized at 4025 g for 2 min. One milliliter of suspension was collected and filtered under vacuum on nitrocellulose filter (pore size: 0.45 μm) and stained with aniline blue. The mass of total mycelia was calculated on the basis of the average values of cross section (9.3×10^{-6} mm^2), density (1.1 g/mL), and dry mass of the hyphae (15% of the wet mass) according to Berg and Söderström (1979). To obtain the fungal fraction of microbial carbon, the values of fungal biomass were converted to fungal carbon (C_{fung}) on the basis of mean values reported for C/N ratio (Killham 1994) and N content (Swift et al. 1979) in fungi. All the physico-chemical and biological analyses on the substrates were carried out in triplicate.

Ecotoxicological analyses

The ecotoxicological analyses were carried out only for substrates collected in 2014 and were performed on both raw and sieved (2 mm) samples. Phytotoxicity tests were performed according to EPA (1996) on a monocotyledon (*Sorghum saccharatum* L.) and a dicotyledon plant (*Lepidium sativum* L.). Ten seeds for each species were placed in Petri dishes, containing an amount of fresh organic amendment equivalent to 10 g of oven-dried organic amendment, and subsequently saturated with water. Standard soil (OECD 1984) and $K_2Cr_2O_7$ were used as negative and positive control, respectively. After incubation in darkness (72 h, 25°C), the number of germinated seeds and total root length were measured.

A test with the ostracod *Heterocypris incongruens* was performed according to Chial and Persoone (2003), evaluating the survival and growth as endpoints.

All ecotoxicological analyses were carried out in three replicates, and the results were expressed as percentages relative to the standard soil (OECD 1984).

Statistical analyses

Two-way analysis of variance (ANOVA) was performed considering as fixed factors the mesocosm typologies (C_p and CP_p) and the sampling time (2004, 2005, and 2014) in order to highlight the differences in each parameter attributable to the substrate and/or sampling time. Normality was assessed using the Shapiro–Wilk test and homoscedasticity using equal variance test. The ANOVAs were followed by the post hoc tests of Holm-Sidak.

The relationships between biological and physico-chemical parameters were evaluated by the Spearman test, according to the non-normal distribution of the data, assessed using the Shapiro–Wilk test.

Statistical analyses and graphical displays were performed by Systat_SigmaPlot_12.2 software (Jandel Scientific, San Jose, California, USA).

RESULTS

The total concentrations of C, N, Cd, Cr, Cu, Ni, and Pb as well as C/N ratios in the substrates of the different mesocosm typologies (control, C; compost with plants, C_p; and mixture of compost and poultry manure with plants, CP_p), at the beginning of the experiment (2004), are reported in Table 1. C and N concentrations ranged between 16.9% and 17.5% d.w. and between 1.57% and 1.63% d.w., respectively. Among the investigated metals, Cu and Pb showed higher

Table 1. Mean values of total concentrations of C and N (% d.w.) as well as Cd, Cr, Cu, Ni, and Pb (μg/g d.w.), and mean C/N ratio in the substrates for mesocosms without plants (C), mesocosms with compost and plants (C_p), and mesocosms with a mixture of compost and poultry manure and with plants (CP_p) in 2004.

2004	C	C_p	CP_p	Legislative Decree 75/2010
C	17.0	17.5	16.9	n.r.
N	1.63	1.63	1.57	n.r.
C/N	10.4	10.7	10.8	Max 50.0
Cd	0.41	0.42	0.31	1.5
Cr	11.8	9.60	9.40	n.r.
Cu	149	139	139	230
Ni	21.3	25.4	22.2	100
Pb	118	101	74.0	140

Notes: Also reported are threshold values for organic amendments for agricultural use as established by current Italian legislation. n.r., The Legislative Decree 75/2010 does not report the threshold values for C, N, and Cr. Only the threshold value for the hexavalent Cr (0.5 μg/g d.w.) is reported.

total concentrations relative to Cd, Cr, and Ni, with values of approximately 100 μg/g d.w.

In 2004, the mean pH values were 7.56 in C and 7.70 and 6.80, respectively, in C_p and CP_p (Fig. 2a), and they significantly increased over time, particularly for the CP_p mesocosm (Appendix S1: Table S1).

In 2004, the mean organic matter content was 41.9% d.w. in C, whereas it was 31.1% d.w. and 39.1% d.w., respectively, in C_p and CP_p (Fig. 2b). Later, the organic matter content in C (39.1% d.w. in 2005 and 22.7% d.w. in 2014) was higher than in C_p (28.8% d.w. in 2005 and 17.9% d.w. in 2014) and CP_p (21.5% d.w. in 2005 and 17.4% d.w. in 2014; Fig. 2b). At each sampling time, no statistically significant differences were observed between the organic matter content in C_p and CP_p (Appendix S1: Table S1). A significant decrease in organic matter occurred only in CP_p, from 2004 to 2005 and from 2004 to 2014 (Fig. 2b; Appendix S1: Table S1).

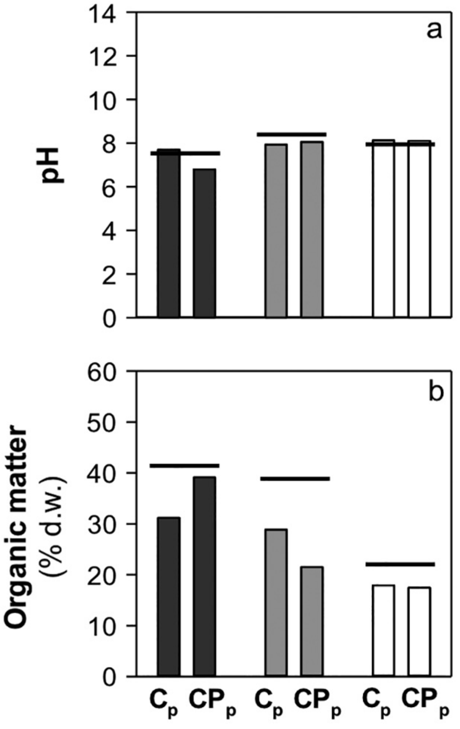

Fig. 2. Mean values (±standard error) of pH (a) and organic matter content (b) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

At the beginning of the experiment, the available fractions of the metals in C were higher than in C_p (Fig. 3), whereas they were higher than those measured in CP_p only for Cd and Pb (Fig. 3a and 3e). Over time, metal availability decreased in C as well as in the mesocosms with plants (C_p and CP_p) reaching at the end of the experiment comparable values in all three types of mesocosm (Fig. 3). The availability of Cd, both in C_p and in CP_p, did not statistically vary from 2004 to 2005, whereas it decreased significantly in 2014 (Fig. 3a; Appendix S1: Table S1); the availability of the other metals already significantly decreased in 2005 both in C_p and in CP_p with the exception of Ni and Pb in C_p (Fig. 3b, e; Appendix S1: Table S1). The greatest decrease in metal availability was observed for the CP_p mesocosms (Fig. 3). In 2004, the available fractions of Cr, Cu, and Ni were significantly higher in CP_p than in C_p (Fig. 3b–d; Appendix S1: Table S1), whereas in 2005 the available fractions of Cd and Pb were significantly higher in C_p than in CP_p (Fig. 3a, e; Appendix S1: Table S1). In 2014, there were no differences in metal availability between the two mesocosm typologies (Fig. 3; Appendix S1: Table S1).

In 2004, the microbial carbon (C_{mic}) in C (0.25 mg/g d.w.) was comparable to that measured in C_p (0.35 mg/g d.w.) and lower than that measured in CP_p (0.52 mg/g d.w.; Fig. 4a). In all the mesocosm typologies, C_{mic} increased over time reaching in 2014 the value of 3.60, 4.79, and 5.37 mg/g d.w., respectively, in C, C_p, and CP_p (Fig. 4a; Appendix S1: Table S1). Besides, for each sampling time, with the exception of 2005 when C_{mic} in CP_p was significantly higher than in C_p, no significant differences were observed between C_p and CP_p (Fig. 4a; Appendix S1: Table S1). At the beginning of the experiment, fungal carbon (C_{fung}) in C was 0.12 mg/g d.w. and decreased over time to 0.03 and 0.01 mg/g d.w., respectively, in 2005 and 2014 (Fig. 4b). C_{fung} in C_p and CP_p decreased over time (Appendix S1: Table S1) from 0.09 mg/g d.w. (Fig. 4b) to values similar to that measured in C (Fig. 4b); at each sampling time (2004, 2005, and 2014), no statistically significant differences were observed between the two typologies (C_p and CP_p) of mesocosms with plants (Fig. 4b; Appendix S1: Table S1).

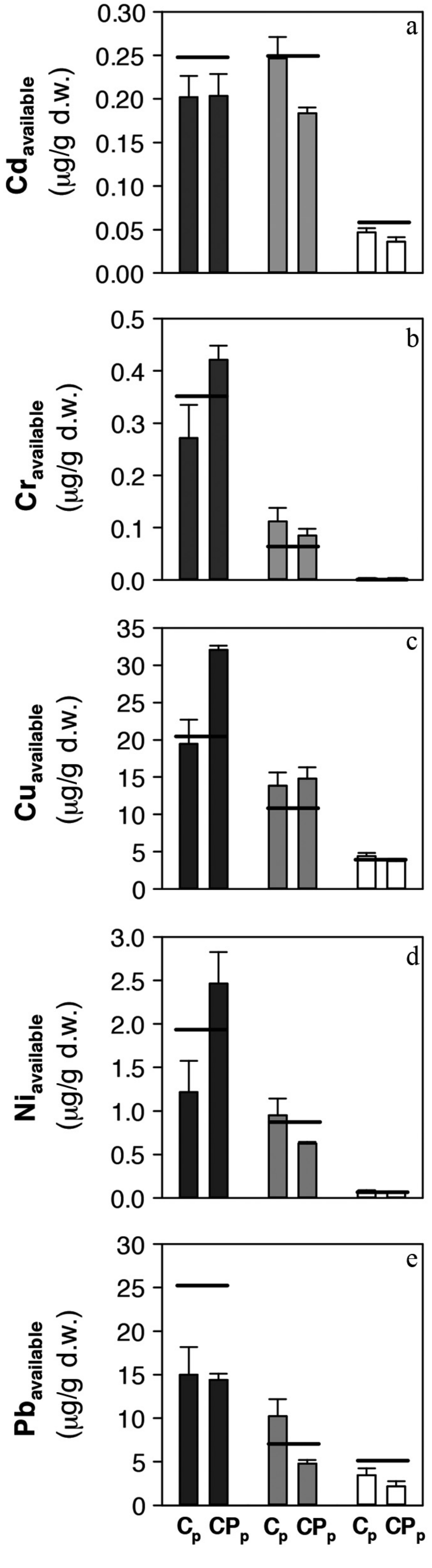

Fig. 3. Mean values (±standard error) of the concentrations of available fractions of Cd (a), Cr (b), Cu (c), Ni (d), and Pb (e) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

The respiration in C was 14.4 mg CO_2 g^{-1} d.w. 10 d^{-1} at the beginning of the experiment and was slightly higher than in C_p and CP_p where it was, respectively, equal to 10.7 and 13.4 mg CO_2 g^{-1} d.w. 10 d^{-1} (Fig. 4c). Respiration in C drastically decreased reaching in 2014 the mean value of 1.25 mg CO_2 g^{-1} d.w. 10 d^{-1} (Fig. 4c). It also significantly decreased in the mesocosms with plants over time (Appendix S1: Table S1) and reached, at the end of the experiment, values comparable to that measured in C mesocosm (Fig. 4c). With the exception of 2004, when the respiration was significantly higher in CP_p than in C_p, in the other sampling times no differences in respiration between these two mesocosm typologies were detected (Fig. 4c; Appendix S1: Table S1). Initially, the metabolic quotient (qCO_2) in C (14.4 mg C–CO_2/mg C_{mic}) was higher than in C_p (10.4 mg C–CO_2/mg C_{mic}) and CP_p (8.07 mg C–CO_2/mg C_{mic}), but over time it drastically decreased, becoming comparable to those detected in the mesocosm with plants (Fig. 4d). A drastic decrease in qCO_2 was observed in C from 2004 to 2005 and 2014 (Fig. 4d), whereas in C_p and CP_p it decreased just from 2005 to 2014 (Fig. 4d; Appendix S1: Table S1). No differences were observed between qCO_2 values in C_p and CP_p (Fig. 4d; Appendix S1: Table S1). In 2004, the mean value of the CEM in C (14.0 mg C–CO_2/g C_{org}) was lower than those calculated for C_p and CP_p (Fig. 4e) and, over time, it decreased reaching the value of 2.57 mg C–CO_2/g C_{org} in 2014 (Fig. 4e). In the mesocosms with plants, CEM decreased in 2014 reaching values of 6.01 and 7.00 mg C–CO_2/g C_{org} in C_p and CP_p, respectively (Fig. 4e), although different temporal trends were observed in the two mesocosm typologies. In fact, in C_p significant differences were observed only between 2004 and 2014, whereas in CP_p they were detected either between 2004 and 2014 or

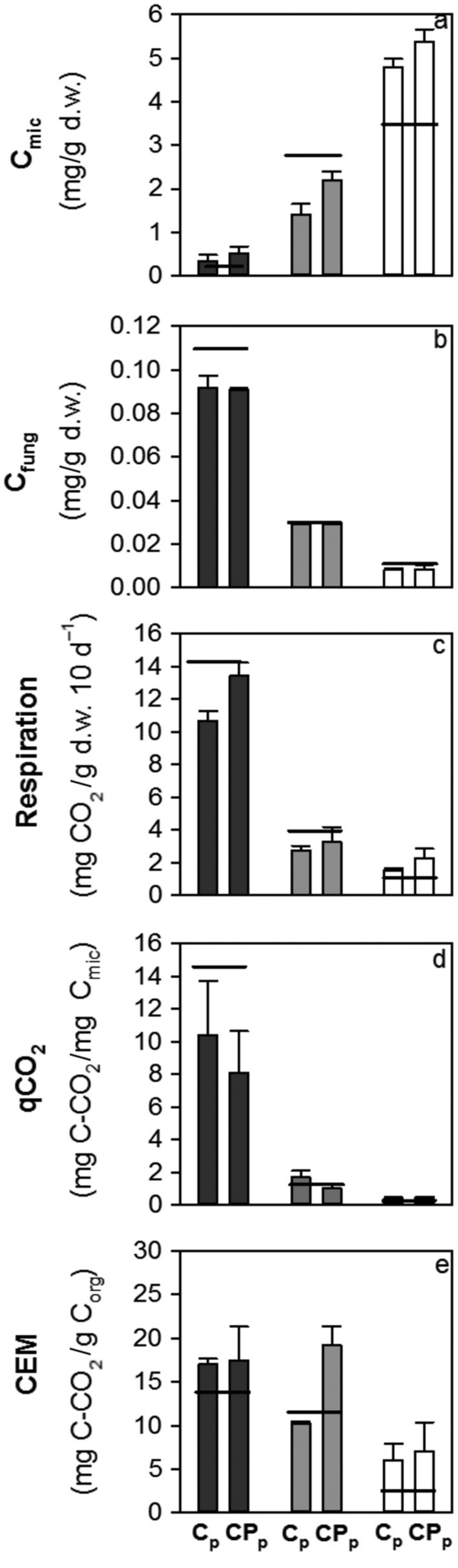

Fig. 4. Mean values (±standard error) of microbial carbon (C_{mic}) (a), fungal carbon (C_{fung}) (b), respiration (Resp) (c), metabolic quotient (qCO_2) (d), and coefficient of endogenous mineralization (e) in mesocosms with compost (C_p) and mesocosms with a mixture of compost and poultry manure (CP_p) in 2004 (black bars), 2005 (gray bars), and 2014 (white bars). The lines indicate the mean value for the mesocosm without plants (C) at the different sampling times.

between 2005 and 2014 (Fig. 4e; Appendix S1: Table S1). Only for the 2005 sampling, significant differences were detected between C_p and CP_p with higher values for CP_p (Fig. 4e).

On the whole, fungal carbon, respiration, and qCO_2 were positively correlated with metal availability and organic matter content, whereas microbial carbon was negatively correlated (Table 2).

The ecotoxicological assays, carried out for the sampling of 2014, showed different responses depending on the tested organisms. The phytotoxicity assays showed percentage effects between 80% and 120% in the substrates of the mesocosm typologies with the exception of root elongation for *Lepidium sativum*, which showed a nearly 150% effect for C_p and CP_p (Fig. 5). The percentage effect of *Heterocypris incongruens* survival was about 86% in C and 60% for C_p and CP_p (Fig. 5); the percentage effect of *H. incongruens* growth was ~65% for C_p and CP_p (Fig. 5). In no ecotoxicological assay, differences were observed between C_p and CP_p.

DISCUSSION

Metal concentrations in the employed amendments were very low, as they did not exceed the threshold values indicated by the National Law (Legislative Decree 75/2010) for organic amendments usable on agricultural soils. Unfortunately, currently the Italian legislation does not consider the acceptable metal thresholds for organic amendments usable to improve degraded or poor soils.

In spite of no differences in total metal concentrations between C_p and CP_p substrates, the poultry manure seems to be the main responsible for the significantly higher Cr, Cu, and Ni availability at the beginning of the experiment (2004), probably because of lower pH. Hernandez et al.

Table 2. Spearman's correlation coefficients assessed between biological and physico-chemical parameters of all the mesocosms.

Parameters	C_{mic}	C_{fung}	Respiration	qCO_2	CEM
pH	0.778***	−0.775***	−0.855***	−0.819***	−0.500*
Organic matter	−0.705***	0.608**	0.604**	0.643**	0.207
$Cd_{available}$	−0.699***	0.595**	0.521*	0.643**	0.243
$Cr_{available}$	−0.874***	0.841***	0.868***	0.878***	0.645**
$Cu_{available}$	−0.856***	0.816***	0.798***	0.839***	0.666**
$Ni_{available}$	−0.874***	0.759***	0.775***	0.827***	0.484*
$Pb_{available}$	−0.903***	0.756***	0.752***	0.847***	0.472*

Note: CEM, coefficient of endogenous mineralization.
$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

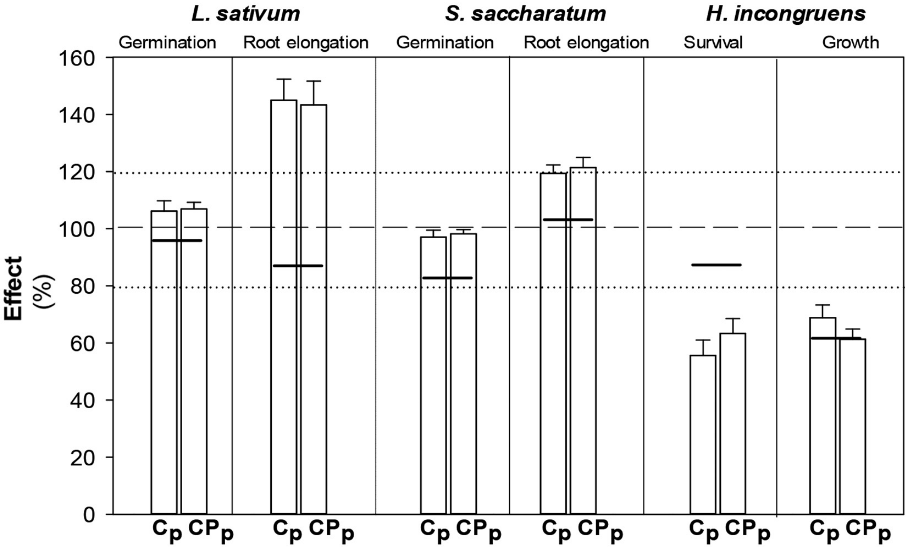

Fig. 5. Mean values of percentage effects of germination and root elongation for *Lepidium sativum* and *Sorghum saccharatum* and percentage effects of survival and growth of *Heterocypris incongruens* for mesocosms with compost and plants (C_p) and mesocosms with a mixture of compost and poultry manure and with plants (CP_p) in 2014. The continuous lines indicate the percentage of effect for mesocosms without plants (C). The dotted line indicates the supposed physiological responses.

(2015) also observed a slight acidification of degraded soils immediately after the addition of peat and manure as amendments, and Pierzynski et al. (1994) report higher metal availability in acidic soils. However, the initially higher metal bioavailability in CP_p substrates did not cause an inhospitable habitat for microorganisms as lower qCO_2, index of stress conditions (Anderson and Domsch 1993), and higher microbial biomass were observed as compared to C_p. Besides, the highest organic matter content in CP_p seems to stimulate the microbial activity, hiding negative effects from higher metal availability.

In the short time, both types of amendment stimulated the microbial biomass as significant increases were observed in comparison with initial values. This effect involved the bacterial rather than fungal component. As fungi are generally more resistant than bacteria to stress conditions (Dighton 2003), the increase in bacterial biomass appears to reflect a sudden improvement in the environmental conditions since the second year after mesocosm setting up, confirmed by the parallel decrease in the respiration rate and qCO_2. In fact, at the beginning of the study period (2004), when the substrate manipulations during the mesocosm setting up occurred, the highest respiration might depend on high energy requirement by microorganisms to survive in adverse conditions (Anderson and Domsch 1993). Nevertheless, the stimulation of respiration caused by higher aeration, which likely occurred during the manipulations, could not be ruled out. Poultry manure augmented the microbial biomass (Garcia-Gil et al. 2000); hence, the mineralization activity remained close to the initial level (Usman et al. 2013), whereas the organic matter content halved from ~40% to 20% d.w. The decrease in the organic matter content and the higher CEM suggest a more efficient degradation of the mixture of compost and poultry manure (Doni et al. 2014, Hernandez et al. 2014, Jain et al. 2014) as compared to the compost alone. Besides, both types of amendment caused a sudden overall decrease in metal availabilities that mainly occurred in the mixture where, since the first year after the mesocosm setting, decreases in Cd, Ni, and Pb were highlighted.

A further change occurred in the biological community in both amended substrates, in the form of a decline in fungal carbon and parallel increase in bacterial carbon, already visible after one year but more pronounced in the long term. Over time, the microbial carbon in both amended substrates even exceeded the values reported for soils collected in a mature maquis in the surroundings of Naples during spring (Marzaioli et al. 2010), that is, under climatic conditions similar to those occurring during the investigated observation period. The inhibitory effects on fungi, in the short and long term, could be due to production of antifungal compounds by bacteria or a better use of resources by bacteria vs. fungi in favorable environmental conditions (Meidute et al. 2008). An improvement of the quality of the substrates, inferable from the steady decrease in the qCO_2 index, already started after one year from mesocosm setting and was likely related to the decrease in metal availability. An overall improvement of substrate quality is also likely to account for the observed changes in respiration. In fact, ten years after mesocosm setting, the respiration had decreased to values typical of agricultural soils of the Mediterranean region amended with compost (Ventorino et al. 2012), suggesting a transition from stressed to unstressed environmental conditions. In the long term (2014), the microbial biomass appeared less active, though more abundant, than in the short term, as the CEM values were lower after ten years than after one year in both amended substrates. This is in line with the small variation in organic matter content between 2005 and 2014, probably due to greater recalcitrance to biodegradation of the remaining organic matter (Maisto et al. 2010).

On the whole, our data are consistent with a regulatory effect of metal availability on microbial biomass and activity. In fact, the fungal carbon appeared to be enhanced and, by contrast, the bacterial carbon appeared to be inhibited by increased metal availability. In addition, the parallel increase in qCO_2 suggests a general stress condition (Anderson and Domsch 1993) that favors fungal rather than microbial biomass that is also provided to organic matter mineralization (Dighton 2003).

The ecotoxicological assays, carried out at the end of the experiment, indicated an overall good quality of the amendments. In fact, the tests more effective in revealing toxicity due to direct contact of the organism with the matrix (germination and root elongation) showed effects linked to individual physiological responses, as they were in the range 80–120%. The biostimulation of root elongation in *Lepidium sativum* reflects a greater sensitivity of this species, as already observed in previous researches (Manzo et al. 2008). By contrast, the ecotoxicity tests revealed non-optimal conditions of the water present in the substrates, as inhibition on both survival and growth of *Heterocypris incongruens* was observed for all the investigated substrates.

A comparison of the data from mesocosms with or without plants shows that, after a sudden effect at the moment of mesocosm setting up, the plants had no major effect in either the short or long term on the parameters investigated (i.e., pH, metal availabilities, fungal carbon, respiration, and qCO_2), with the exception of microbial biomass and CEM. In fact, plants seem to slowly enhance the mineralization of organic matter by microorganisms (Bardgett and Wardle 2003 Van Elsas et al. 2006) as after ten years from mesocosm setting, on average, a twofold increase in CEM and a 1.2-fold decrease in organic matter content were observed in C_p and CP_p relative to C. In addition, an improvement of the substrate quality linked to a reduction in metal availabilities was clearly visible (in 2004, Pb available fractions in C_p or CP_p were ~1.7-fold lower than in C). As the highest decreases in Cd and Pb availabilities were observed in mesocosms with plants (C_p and CP_p), root uptake and accumulation of these metals could not be ruled out (Tangahu et al. 2011, Yoon et al. 2006, Gupta et al. 2014).

In conclusion, the results of the present investigation show that a single application of organic amendments enhanced the microbial biomass, in both the short (one year) and long term (ten years). The data also suggest the bacterial component to be more competitive than the fungi in the long term. The progressive reduction in qCO_2 (a stress index) and respiration (energy requirement) levels observed during the ten-year experiment suggests that initially high stress conditions reduced with time. The applied amendments appeared to be more favorable to microbial growth in the long than short term, despite the long-term reduction in the mineralization rate likely due to the chemical complexity of residual organic matter. Ten years after mesocosm setting, the metal availability drastically decreased. The addition of poultry manure to compost improved chemical and biological parameters especially in the brief time. In the long term, the single application of either of the investigated amendments showed low ecotoxicity, thus confirming their suitability for reducing the impact of limestone waste on the territory.

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