CSIRO PUBLISHING
Soil Research
https://doi.org/10.1071/SR19373

Soil biochemical activities after the application of pyroligneous acid to soil

Roberto Cardelli[A,B], *Michelangelo Becagli*[A], *Fausto Marchini*[A], *and Alessandro Saviozzi*[A]

[A]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy.
[B]Corresponding author. Email address: roberto.cardelli@unipi.it

Abstract. Pyroligneous acid (PA) is produced during the combustion of woody biomass and is a complex aqueous fraction resulting from the thermochemical rupture of the components of vegetable biomass. We evaluated the effect of PA on the soil microbial community and activity in order to assess the applicability of this acid in soil and to gather further information on the mechanisms of its toxicity or stimulation. Five concentrations of PA solution (0, 0.5, 1, 2 and 5%) were selected to monitor the biochemical parameters of the soil. The respirometric test showed that the increase in the evolved carbon dioxide-carbon (C) was not due to a release of the native organic C from the soil, but only from the organic compounds of PA. The highest values of microbial biomass content were found in the soil treated with the lowest PA doses, but decreased with increased doses. At higher application doses (2 and 5%), there was a decrease in most enzymatic activities and a loss of soil quality. When PA was applied in doses of up to 1%, our results indicated no negative effects on soil biology and that there was even an improvement.

Additional keywords: soil enzymes, soil microbial biomass, soil respiration, wood vinegar.

Received 16 December 2019, accepted 10 March 2020, published online 22 April 2020

Introduction

The global production of woody biomass produced by forestry has been estimated at 3.8 billion m^3 per year (FAO 2015). Its combustion degrades the soil and releases into the atmosphere a large number of particles, volatile organic carbon (C) and semi-volatile organic C compounds, ash, sulfate aerosols and trace gases. These pollutants increase greenhouse gas emissions, which can contribute to many serious environmental problems on an overall scale such as the increase in global climate change, the extinction of biodiversity as well as serious socioeconomic and health problems. Therefore, it is important to minimise combustion or waste plant biomass and, instead, to develop means of reducing pollution at a low cost along with sustainable technologies to convert it into useful bioproducts.

Pyrolysis is a thermochemical process that leads to the thermal degradation of materials in the absence or near absence of oxygen (Balat *et al*. 2009). During slow pyrolysis, the organic bonds are decomposed, and the plant biomass is converted into organic vapours and solid charcoal. The gaseous products of pyrolysis are water vapour, tar and volatiles that are condensed and collected using filters and cold traps (Mansur *et al*. 2013). The condensed organic vapours form an aqueous liquid-fraction rich in oxygenated compounds (Mathew *et al*. 2014) called pyroligneous acid (PA) or wood vinegar.

The PA is a complex aqueous liquid fraction that results from the thermochemical rupture of the components of the plant biomass such as cellulose, hemicellulose and lignin. The PA is produced during the combustion of woody biomass, when the gases from the oven are channelled so as to allow the condensation of the steam.

PA is a yellowish brown or dark brown liquid usually including a complex mixture of different classes of organic compounds, namely aldehydes, ketones, alcohols, organic acids, esters, furan and pyran derivatives, phenols, hydrocarbons and nitrogen (N) compounds, in which the main ones are organic and phenolic acids (Souza *et al*. 2012). The high concentration of acids (up to 25% by weight) gives PA a low pH (pH < 3). These substances may have positive effects if correctly applied in terms of quantity and application time.

Some studies have been carried out to verify the effects of PA on soil and plants (Prasertsit *et al*. 2011; Kang *et al*. 2012; Souza *et al*. 2012; Rui *et al*. 2014; Benzon *et al*. 2015). The PA is used in various areas, such as an antioxidant, antimicrobial, anti-inflammatory, plant growth stimulator, coagulant for natural rubber and termiticidal agent and pesticide (Oramahi *et al*. 2018). The results obtained by Mmojieje and Hornung (2015) demonstrate the benefits of PA through its application as a pesticide against mites and red spiders at a 10% dilution, suggesting a potential role in crop protection. Furthermore, in the same work a phytotoxic effect was reported on its application in dilutions above 20%.

As PA is used to control pests and diseases, we hypothesise that toxic effects might reduce the soil microbial population size, activity and population growth, at least in the most concentrated doses. Koc *et al*. (2019) confirmed that wood

vinegar could be used safely as a pesticide and that the increase in soil enzyme activity and productivity may indicate a positive effect on crop production.

Soil biochemical activities are commonly used as indicators of soil quality as they are more sensitive to changes in management than the physical or chemical properties of the soil – they measure the key microbial reactions involved in the soil nutrient cycles and can be easily measured (Paz-Ferreiro and Fu 2016). However, there are very few publications on the effects of adding PA on soil enzyme activities. We thus selected various biochemical properties for analysis based on their importance in the decomposition of organic matter, and several enzymes involved in different nutrient cycles were finally chosen.

We calculated the geometric mean (GMea) of enzyme activities in order to estimate soil quality, considering that it is sensitive to changes in soil management (García-Ruiz *et al.* 2008). We also monitored soil basal respiration and microbial biomass in order to assess the effects of management practices (Steiner *et al.* 2004) or toxic agents (Beck and Bengel 1992) on the soil microbial community.

We assessed the effects of PA on the soil microbial community in order to evaluate the applicability of PA to soil and to gather further information on the mechanisms of its toxicity or stimulation. In fact, many researchers have shown that PA enhances harvest yield of many plant species through enhanced seed germination, plant growth, fruit size, fruit weight and quality of many fruits and vegetables (Zulkarami *et al.* 2011).

The starting hypothesis of this research is that the PA has a positive effect on soil quality, if applied in appropriate doses. The aim of this work is to investigate whether PA can improve soil quality by verifying the effects on microbial activity at various application doses.

Materials and methods

Soil

The surface (0–15 cm) of a loamy sand soil, classified as Typic Xerorthent (USDA Soil Taxonomy), was collected from a dedicated agricultural area at Pontasserchio, located ~9 km from the sea (43°45′51″N, 10°23′23″E) and 1 m above sea level (Pisa, Italy). The soil samples were collected in March 2019 and consisted of 10 cores measuring 5 cm diameter × 15 cm depth. These were air-dried and passed through a 2-mm sieve to remove large residue fragments. The main soil characteristics were determined by standard methods (SISS 1995): 77% sand (2–0.05 mm), 14% silt (0.05–0.002 mm) and 9% clay (<0.002 mm) by the pipette method; pH 7.9; 40.0 cmol kg^{-1} H^+ hydrolytic acidity by titration of 0.5 M calcium acetate extract (ratio 1 : 2.5) with 0.1 M NaOH; 10.0 g kg^{-1} total organic C (TOC) by dry combustion (induction furnace 900 CS, Eltra); 1.03 g kg^{-1} total N by the Kjeldahl procedure after acid digestion; 16.4 mg kg^{-1} available P measured in 0.5 N $NaHCO_3$ extract at pH 8.5; 43.8 mg kg^{-1} available K determined in 1 N CH_3COONH_4 extract at pH 7.0; 13.7 cmol (+) kg^{-1} cation exchange capacity (CEC) determined by Ba^{2+} saturation and subsequent complete replacement of Ba^{2+} with Mg^{2+}; 89% base saturation calculated as the percentage of CEC occupied by base cations; and 44% maximum water-holding capacity.

Pyroligneous acid

The PA, also called wood vinegar or wood distillate, was produced by RM Impianti srl (Arezzo, Italy) and was obtained from native forest plant essences with the same physiological water through pyrolysis. The manufacturer recommends using the product in the open field by a fertigation system at a 0.5% dilution. We tested higher doses of the product (up to 5%) to investigate the effects on the biochemical activities of the soil. Previous work verified the chemical composition of PA, which is quite variable depending on the starting woody material and the production temperature (Mathew and Zakaria 2015; Grewal *et al.* 2018). The main characteristics of PA used in this study follow: pH 2.8, density 1.037 g mL^{-1}, total organic C 33.8 g L^{-1}, total N 0.43 g L^{-1}, organic acid 3.23%, phenolic compounds 13.0 g L^{-1} and methanol 13.4 g L^{-1}. Polychlorobiphenyls and polycyclic aromatic hydrocarbons (PAHs) were also determined, but none of these toxic compounds were present in relevant concentrations. Among PAHs, only acenaphthylene and phenanthrene reached 0.09 ng L^{-1}, well below the most restrictive legislative limits.

Treatments of soil with PA

In 250-mL microcosms, the experiment was conducted in triplicate with five treatments (15 microcosms total) to differentiate between the influence of PA doses. Five PA dilutions were selected to monitor the soil parameters: control only water, low (L) 0.5%, medium-low (ML) 1%, medium-high (MH) 2% and high (H) 5% doses. The samples were watered with the different solutions at the 60% maximum water-holding capacity (26.4 ml · 100 g^{-1}), which we considered to be optimal for soil biological activities. They were then sealed with Parafilm to permit gaseous exchange, and incubated at 25 ± 1°C for 10 days. After 10 days, the samples were refrigerated at 4°C for the analyses.

Analyses

A short-term (21 days) aerobic incubation was used to determine the potential of the samples to mineralise organic C. The carbon dioxide (CO_2) evolution was monitored daily during days 1–21: 50 g of soil was placed in 250-mL glass containers sealed with rubber stoppers, moistened with 13.2 mL of the various solutions of PA, at 60% of the maximum water-holding capacity, and incubated at 25 ± 1°C; the CO_2 evolved was trapped in NaOH solution and the alkali excess was titrated with HCl (Levi-Minzi *et al.* 1990). The results, normalised with respect to time, were expressed as mg of C mineralised · 100 g^{-1} of dry soil.

Soil microbial biomass C (MB-C) was determined according to Vance *et al.* (1987) with the extraction of organic C from fumigated and unfumigated soils by 1 N K_2SO_4. The organic C was then measured using a QBD1200 Laboratory TOC Analyser (Hach Co., Loveland, CO, USA). An extraction efficiency coefficient (K_c) of 0.45 was used to convert the difference in soluble C between the fumigated and unfumigated soils into microbial biomass C.

Specific respiration of biomass (qCO_2) was calculated as follows: the CO_2 evolved during the 15th day of incubation (Fig. 1) was used as basal respiration value because, after that period, the soil reached a constant rate of CO_2 production. The qCO_2 (μg CO_2-C basal h^{-1} μg biomass C^{-1}) represents the microbial respiration per biomass unit (Schnurer *et al*. 1985).

Dehydrogenase (Deh) activity was assayed following Tabatabai (1994), based on a colorimetric assay at 488 nm of 1,3,5-triphenylformazan (TPF) produced by the microorganism reduction of 2,3,5-triphenyltetrazolium chloride; Deh activity was expressed as μmol of TPF g^{-1} soil h^{-1}.

The β-glucosidase (Glu) activity was assayed by a colorimetric method, using 4-nitrophenyl-β-D-glucopiranosyde as a substrate: soil samples were incubated at 37°C for 60 min. The reaction product p-nitrophenol was determined at 410 nm (Eivazi and Tabatabai 1988) and Glu activity was expressed as μmol of p-nitrophenol g^{-1} soil h^{-1}.

Following Eivazi and Tabatabai (1977), alkaline phosphatase (Pal) activity was based on the hydrolysis of p-nitrophenyl phosphate added to soil samples. This phosphate releases p-nitrophenol, which can be detected colourimetrically at 410 nm and Pal activity was expressed as μmol of p-nitrophenol g^{-1} soil h^{-1}.

Arylsulfatase (Aryl) activity was determined by a colourimetric method, using p-nitrophenyl sulfate as a substrate: soil samples were incubated at 37°C for 1 h and the reaction product (p-nitrophenol) was extracted by dilute alkali (0.5 M $CaCl_2$ and 0.5 M NaOH) and determined at 400 nm (Tabatabai and Bremner 1970); Aryl activity was expressed as μmol of p-nitrophenol g^{-1} soil h^{-1}.

The GMea (a general index to integrate information from variables that possess different units and range of variation) of the assayed enzyme activities was calculated for each sample as follows: $GMea = (Deh \times Glu \times Pal \times Aryl)^{1/4}$ (Paz-Ferreiro *et al*. 2012).

The hydrolysis rate of fluorescein diacetate (FDA) was estimated as reported by Dick *et al*. (1996), by determining the concentration of fluorescein released by FDA (μg g^{-1} $2h^{-1}$) at 490 nm.

The determination of catalase (Cat) activity was based on the rates of recovery of H_2O_2 by titration with $KMnO_4$ in the presence of sulfuric acid (Jin *et al*. 2009); Cat activity was expressed as μmol of $KMnO_4$ g^{-1} soil h^{-1}.

Urease (Ur) activity was determined according to Kandeler and Gerber (1988), based on the spectrophotometric measurement of released ammonia after a 2-h incubation of soil samples with urea substrate at 37°C; Ur activity was expressed as μg of NH_4^+-N g^{-1} soil $2h^{-1}$.

Statistical analysis

Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analysis. Data were expressed on the basis of the oven-dry weight of the soil. Results were the means of determinations carried out on three replicates. A simple linear regression analysis was used to determine the relationship between the cumulative C loss and the PA doses applied to soil. The differences between treatments were analysed using a one-way ANOVA and cluster analysis. Significantly different means were separated at $P < 0.05$ using Tukey's test (Steel *et al*. 1997).

Results and discussion

The pH of the soil with the addition of the H dose of PA dropped by only 0.9 points compared with the control (from 7.9 down to 7.0, data not shown). We believe that this limited variation does not explain any changes in the properties that we discuss below.

Effects of PA on soil respiration

The daily emission of CO_2 (Fig. 1) on the first day showed that the H treatment of PA induced a low production of CO_2, indicating a temporary stress of the microflora immediately after the PA addition. From the second day, the pattern showed a clear influence of PA on CO_2 emission, with values that were constantly higher than the other treatments throughout the whole incubation period (21 days), as a result of a greater amount of mineralisable organic C. Thus, the H dose had an initial inhibiting effect on the activity of microbial biomass, probably due to the high concentration of phenolic compounds and organic acids of PA (Grewal *et al*. 2018); in fact, at certain doses PA is an effective antimicrobial solution (Lee *et al*. 2011).

The cumulative emission of CO_2 from PA–soil systems is shown in Fig. 2. The curves suggest that the positive effects on

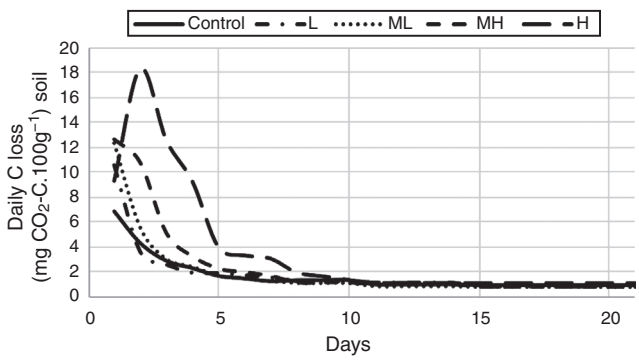

Fig. 1. Daily C loss over 21-day incubation in PA–soil systems at different application rates. L, low; ML, medium-low; MH, medium-high; and H, high.

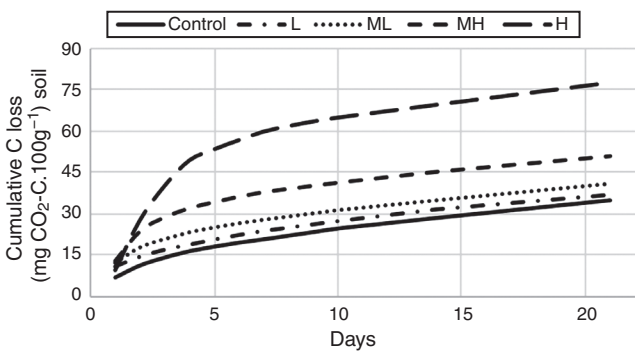

Fig. 2. Cumulative C loss over 21-day incubation in PA–soil systems at different application rates. L, low; ML, medium-low; MH, medium-high; and H, high.

C mineralisation were due to the incremental addition of PA and the difference between the five treatments was the quantity of organic matter added to soil. The addition of readily available substrate following the incorporation of the different doses of PA either stimulated microbial activity or put it under stress. In fact, the highest C mineralisation was for the H treatment (77.66 mg CO_2-C · 100 g^{-1}, Table 1). After the first phase of toxicity and inhibition, revealed by the patterns shown in Fig. 1, in the H treatment a resiliency evidently followed, which stimulated the activity of the remaining biomass.

The regression line describing the cumulative C loss from PA–soil systems as a function of different loading rates of PA-C was calculated ($y = 33.09 + 0.9909x$, $R^2 = 0.996$). This enabled us to estimate, without having to use isotopically labelled biochar, the influence of the material on the native organic C decomposition in the soil. This procedure consists of comparing the value of the intercept of the regression line (Fig. 3) with the *y*-axis, corresponding to CO_2-C produced at the application of 0 g 100 g^{-1} TOC, with the CO_2-C actually measured in the control (Levi-Minzi *et al*. 1990). The value of the priming ratio (0.95), obtained by dividing the CO_2-C value of 33.09 mg 100 g^{-1} estimated by the intercept of the line with the *y*-axis by the value of CO_2-C of 34.80 mg 100 g^{-1} actually measured in the control, indicates a negative priming effect following the PA addition, i.e. a reduction in the microbial decomposition of native organic C. This result indicates that the higher values of enhanced CO_2-C in the treated samples were not due to a C release of native soil organic matter (Cardelli *et al*. 2016).

Table 1. CO_2-C evolved after 21 days, soil microbial biomass C and specific respiration of biomass (qCO_2) in the different treatments of soil
Means followed by the same letter in a column do not significantly differ ($P < 0.05$) according to Tukey's test. L, low; ML, medium-low; MH, medium-high; and H, high

Treatment	C mineralised (mg CO_2-C 100 g^{-1} soil)	Biomass C (μg C g^{-1} soil)	qCO_2 (μg CO_2-C h^{-1}μg biomass $C^{-1} \times 10^2$)
Control	34.80	660.23 b	5.74 bc
L	36.54	773.88 a	4.09 c
ML	41.04	721.95 a	4.96 bc
MH	50.58	582.23 bc	5.80 b
H	77.66	495.57 c	9.67 a

Effects of PA on soil biomass

Values of MB-C ranged within 495–773 μg g^{-1} of soil (Table 2). The highest values of MB-C were found in the L and ML soil, treated with the lowest doses of PA. This is in line with results of Rui *et al*. (2014), who reported promoting effects on total microbial quantities exhibited by soils treated with low doses (0.3%) of PA. In our work, MH and H presented lower quantities of microbial biomass, probably due to a repressive dose-effect of the toxic components of PA, such as phenolic compounds and organic acids. Also Du *et al*. (2016) found that contents of microbial biomass carbon decreased in a sandy soil with increasing doses of wood vinegar.

If the basal respiration rates are related to the biomass size, the qCO_2 thus obtained represents the CO_2-C produced per unit biomass and time. This parameter indicates how efficiently the microbial biomass uses available C for biosynthesis rather than for maintenance of respiration. Anderson and Domsch (1993) reported that qCO_2 increases when the ecosystem is stressed, polluted or in adverse climatic conditions.

In our study, the qCO_2 was influenced by the different doses of PA and showed an inverse relation with the biomass content (Table 2). The highest and lowest values of qCO_2 were in H and L soil respectively, with 9.67 and 4.09 μg CO_2-C basal h^{-1} μg biomass C^{-1}. The high qCO_2 value of H suggests intense

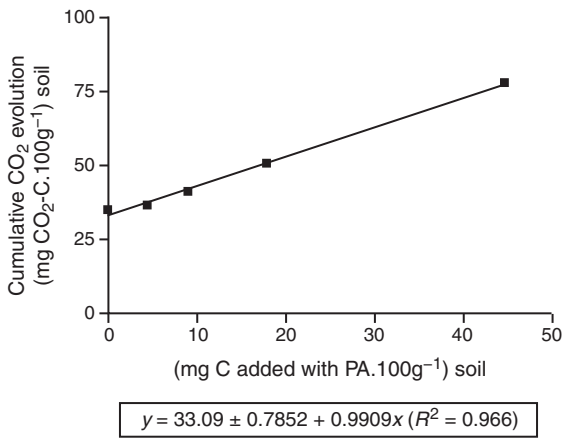

Fig. 3. Cumulative evolution of CO_2-C from soil systems as a function of different application rates of PA.

Table 2. Dehydrogenase, β-glucosidase, alkaline phosphatase, arylsulfatase and geometric mean index (GMea) in the different treatments of soil
Means followed by the same letter in a column do not significantly differ ($P < 0.05$) according to Tukey's test. L, low; ML, medium-low; MH, medium-high; and H, high

Treatment	Dehydrogenase (μmol TPF $g^{-1}h^{-1}$)	β-glucosidase	Phosphatase	Arylsulfatase (μmol p-nitrophenol $g^{-1}h^{-1}$)	GMea
Control	0.126 a	0.425 a	1.486 a	0.224 a	0.365 a
L	0.122 a	0.406 ab	1.535 a	0.209 ab	0.355 a
ML	0.123 a	0.418 a	1.720 a	0.216 ab	0.372 a
MH	0.081 b	0.392 b	1.592 a	0.201 bc	0.317 b
H	0.084 b	0.387 b	1.691 a	0.180 c	0.315 b

competition for the available C after the treatment. Moreover, Islam and Weil (2000) reported that perturbed systems, such as H, favoured bacteria with a low efficiency in C assimilation, while more efficient fungi tended to dominate in natural non-stressed systems. It is interesting to note that the low microbial pool size of H (Table 2) corresponded to the highest level of qCO_2, and a high amount of microbial biomass of L soil corresponded to the lowest qCO_2. These results suggest, in line with Nsamibana *et al*. (2004), the predominance of more active microorganisms in H soil. Anderson and Domsch (1993) also found an inverse relation between qCO_2 and soil reaction. Accordingly, in our work the highest qCO_2 was in the most acidic soil of the H treatment (data not shown).

Effects of PA on soil enzyme activities

The Deh activity is an important component of the enzymatic system of every microorganism. A significantly lower Deh activity was found in the MH and H compared with control, L and ML doses (Table 2). This suggests that the microorganisms are unable to use PA as a substrate because they were inhibited by the toxic effect of phenolic compounds and volatile acids, which are abundant in PA.

However, addition of PA up to 1% dilution did not affect Deh activity compared with the control. Given that PA causes significant increases in the quantities of bacteria in soils, but does not change the characteristics of the soil bacterial composition (Rui *et al*. 2014), the higher respiration values coupled with lower Deh activity in H may indicate that the soil microorganisms were less efficient under high doses of PA. This behaviour was also true for Glu and Aryl activities, but Pal activity seemed unaffected. In fact, Pal activity exhibited no difference among the treatments analysed (Table 2). This result indicates that P cycling in the used soil was unaltered by addition of PA. Koc *et al*. (2018) found that wood vinegar did not affect Pal activity in wheat agro-ecosystems, and it was even promising in increasing the enzyme activity in some applications. Lu *et al.* (2015) found that Pal activity was significantly improved in bulk and rhizosphere of maize after biochar–manure compost and PA solution amendment. To the best of our knowledge there are few studies on the effect of PA addition to soil on these enzyme activities to compare our data with.

The GMea has proved to be a good index for estimating soil quality as its values are related to other physical, chemical and biological properties of soil (García-Ruiz *et al*. 2008). In our study, GMea statistically differed in the MH and H compared with the other treatments. The lower GMea values in MH and H suggest that the high doses of PA were harmful for the soil microorganisms and resulted in a decrease in soil quality. The L and ML had higher values of GMea than the MH and H doses, indicating that the quality of soil was preserved.

The rate of hydrolysis of FDA by soil samples has been considered as an index of the overall microbial activity (Schnurer and Rosswall 1982), because this hydrolysis is carried out by active cells and is due to a variety of enzymes. There was no noticeable influence of the doses of PA on FDA in the L, ML and MH treatments, but there was a clear decrease in H (Table 3). This confirms the GMea results regarding a general decrease in soil quality, but only when the H dose was used.

The addition of PA to soil did not affect Cat activity in any of the treatments (Table 3), probably as a result of the increase in the biologically-active remaining microflora.

The Ur activity was very high only for the highest dose (H), but was lower for the L, ML and MH doses. The light soluble organic material contributed by the PA and exploitable by Ur could explain the increase in activity in the H treatment. This is confirmed by Sun *et al*. (2020) in a study evaluating the impact of wood vinegar on ammonia volatilisation from N-fertilised rice paddy soil considering Ur activity: in comparison to the control, the two wood vinegar-only treatments increased the NH_4^+-N concentrations of the topsoil and floodwater as a result of the possibly stimulating effect of wood vinegar on Ur activity.

As mentioned regarding Pal activity, there are no other reported works on the effect of PA addition to soil for Ur activity, and several hypotheses could possibly explain the behaviour of this enzyme which also occurs in an extracellular form.

Table 3. FDA-ase, catalase and urease activities in the different treatments of soil

Means followed by the same letter in a column do not significantly differ ($P < 0.05$) according to Tukey's test. L, low; ML, medium-low; MH, medium-high; and H, high

Treatment	FDA-ase (µg fluorescein g^{-1} $2h^{-1}$)	Catalase (µmol $KMnO_4$ $g^{-1}h^{-1}$)	Urease (µg NH_4^+-N g^{-1} $2h^{-1}$)
Control	96.20 a	288.9 a	14.07 b
L	95.65 a	283.4 a	11.68 c
ML	99.41 a	282.5 a	11.28 c
MH	102.50 a	281.4 a	10.21 c
H	55.08 b	280.7 a	18.71 a

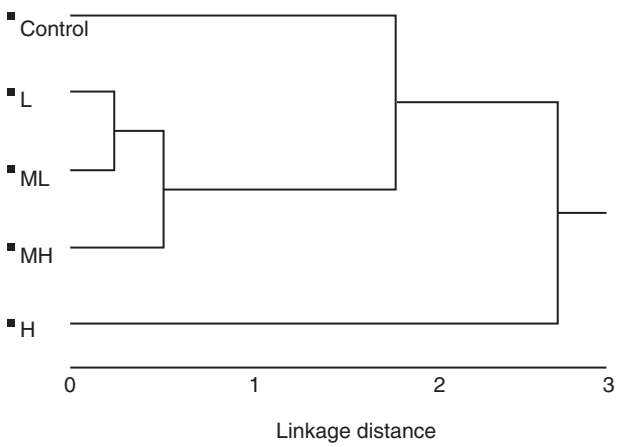

Fig. 4. Horizontal dendrogram based on the average linkage for the different study doses. Considered variables: FDA-ase, catalase and urease activities (Table 3). L, low; ML, medium-low; MH, medium-high; and H, high.

Cluster analysis was used to evaluate the underlying structure in the data of three enzymatic activities (FDA-ase, Cat and Ur). The dendrogram of the study doses based on the average linkage is shown in Fig. 4. At cluster and sub-cluster level, the soil treated with H dose, which recorded the lowest enzymatic activity (FDA-ase) and the highest (Ur) (Table 3), was alone in one cluster. The intermediate doses (L, ML and MH) were close together showing similar effects on soil and good enzymatic activities compared with the untreated soil. Overall, the dendrogram reveals that all the soils treated with PA differed from the control, but only the H dose had marked dissimilar behaviour.

Conclusions

The respirometric test showed a slight negative priming effect and that the increase in CO_2-C in the treated samples was not due to a C release from the native soil organic matter but to the mineralisation of PA organic C. This implies that the application of PA to the soil did not accelerate the decomposition of the native organic matter.

We found that the microbial biomass decreased quantitatively with increasing doses of PA, but the C-assimilation efficiency of the remaining part improved.

At higher application doses (2 and 5% dilution) there was a decrease in most of the enzymatic activities. The GMea also indicated a loss of soil quality at these doses. The Pal and Cat activities were not affected by addition of PA to soil, but Ur appeared to be stimulated.

Our results indicate that PA had no negative effects on the soil and that there was even an improvement, but only up to doses of 1% dilution. In our opinion this quantity could be recommended by the producers of the PA, although it will require further investigation to allow the development of this product on a commercial scale.

Further studies are needed to investigate the application of PA to soil and the influence of PA on agriculture, as this type of material could prove to be of great interest for the sustainability of cropping systems.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors acknowledge RM IMPIANTI srl Energy Solutions (Arezzo, Italy) for having produced and provided the wood distillate.

References

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) a specific activity parameter to assess the effect of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology & Biochemistry* **25**, 393–395. doi:10.1016/0038-0717(93)90140-7

Balat M, Balat M, Kırtay E, Balat H (2009) Main routes for the thermoconversion of biomass into fuels and chemicals. Part 1: pyrolysis systems. *Energy Conversion and Management* **50**, 3147–3157. doi:10.1016/j.enconman.2009.08.014

Beck T, Bengel A (1992) Die mikrobielle Biomasse in Böden, Teil I: Ihre Bestimmung, Indikatorfunktion und Bedeutung für die Stoffumsetzungen. SuB Heft 01/92, III-6-III-10.

Denevan WM (1996) A bluff model of riverine settlement in prehistoric Amazonia. *Annals of the Association of American Geographers* **86**, 654–681.

Benzon HRL, Rubenecia MRU, Ultra VU Jr, Lee SC (2015) Chemical and biological properties of paddy soil treated with herbicides and pyroligneous acid. *The Journal of Agricultural Science* **7**, 20–29.

Cardelli R, Becagli M, Marchini F, Saviozzi A (2016) Short-term releases of CO_2 from newly mixed biochar and calcareous soil *Soil Use and Management* **32**, 543–545. doi:10.1111/sum.12306

Dick RP, Breakwell DP, Turco RF (1996) Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In 'Methods for Assessing Soil Quality'. (Eds JW Doran, AJ Jones) pp. 247–271. (SSSA: Madison WI, USA)

Du W, Zhu Y, Zhang X, Geng Y, Lin P (2016) Effects of wood vinegar on microbial biomasses and enzyme activity in sandy soil. *Shuitu Baochi Tongbao* **3**, 358–368.

Eivazi F, Tabatabai MA (1977) Phosphatases in soil. *Soil Biology & Biochemistry* **9**, 167–172. doi:10.1016/0038-0717(77)90070-0

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. *Soil Biology & Biochemistry* **20**, 601–606. doi:10.1016/0038-0717(88)90141-1

FAO (2015) 'Global Forest Resources Assessment 2015.' (Food and Agriculture Organization of the United Nation: Rome)

García-Ruiz R, Ochoa V, Hinojosa MB, Carreira JA (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology & Biochemistry* **40**, 2137–2145. doi:10.1016/j.soilbio.2008.03.023

Grewal A, Abbey L, Gunupuru LR (2018) Production, prospects and potential application of pyroligneous acid in agriculture. *Journal of Analytical and Applied Pyrolysis* **135**, 152–159. doi:10.1016/j.jaap.2018.09.008

Islam KR, Weil RR (2000) Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. *Agriculture, Ecosystems & Environment* **79**, 9–16. doi:10.1016/S0167-8809(99)00145-0

Jin K, Sleutel S, Buchan D, De Neve S, Cai DX, Gabriels D, Jin JY (2009) Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. *Soil & Tillage Research* **104**, 115–120. doi:10.1016/j.still.2009.02.004

Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* **6**, 68–72. doi:10.1007/BF00257924

Kang MY, Heo KH, Kim JH, Cho SS, Seo PD, Ric CM, Lee SC (2012) Effects of carbonized rice hull and wood charcoal mixed with pyroligneous acid on the yield, and antioxidant and nutritional quality of rice. *Turkish Journal of Agriculture and Forestry* **36**, 45–53.

Koc I, Yardim EN, Akca MO, Namli A (2018) Impact of pesticides and wood vinegar, used in wheat agro-ecosystems, on the soil enzyme activities. *Fresenius Environmental Bulletin* **27**, 2442–2448.

Koc I, Ogun E, Namli A, Mendes M, Kutlu E, Yardim EN (2019) The effects of wood vinegar on some soil microorganisms. *Applied Ecology and Environmental Research* **17**, 2437–2447. doi:10.15666/aeer/1702_24372447

Lee CS, Yi EH, Kim HR, Huh SR, Sung SH, Chung MH, Ye SK (2011) Anti-dermatitis effects of oak wood vinegar on the DNCB-induced contact hypersensitivity via STAT3 suppression. *Journal of Ethnopharmacology* **135**, 747–753. doi:10.1016/j.jep.2011.04.009

Levi-Minzi R, Riffaldi R, Saviozzi A (1990) Carbon mineralization in soil amended with different organic materials. *Agriculture, Ecosystems & Environment* **31**, 325–335. doi:10.1016/0167-8809(90)90231-2

Lu H, Lashari MS, Liu X, Ji H, Li L, Zheng J, Kibue GW, Joseph S, Pan G (2015) Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China. *European Journal of Soil Biology* **70**, 67–76. doi:10.1016/j.ejsobi.2015.07.005

Mansur D, Yoshikawa T, Norinaga K, Hayashi J, Tago T, Masuda T (2013) Production of ketones from pyroligneous acid of woody biomass pyrolysis over an iron-oxide catalyst. *Fuel* **103**, 130–134. doi:10.1016/j.fuel.2011.04.003

Mathew S, Zakaria ZA (2015) Pyroligneous acid - the smoky acidic liquid from plant biomass. *Applied Microbiology and Biotechnology* **99**, 611–622. doi:10.1007/s00253-014-6242-1

Mathew S, Zakaria ZA, Musa F, Ngadiran S, Suan CL (2014) Free radical scavenging property and chemical profile of pyroligneous acid from pineapple waste biomass. Presented at the 5th International Conference on Wellbeing for Biotechnology, 11–12 June, UTM Kuala Lumpur, Malaysia. pp. 1985–1992.

Mmojieje J, Hornung A (2015) The potential application of pyroligneous acid in the UK agricultural industry. *Journal of Crop Improvement* **29**, 228–246. doi:10.1080/15427528.2014.995328

Nsamibana D, Haynes RT, Wallis FM (2004) Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. *Applied Soil Ecology* **2**, 81–92.

Oramahi HA, Yoshimura T, Diba F, Setyawati D (2018) Antifungal and antitermitic activities of wood vinegar from oil palm trunk. *Journal of Wood Science* **64**(3), 311–317. doi:10.1007/s10086-018-1703-2

Paz-Ferreiro J, Fu S (2016) Biological indices for soil quality evaluation: perspectives and limitations. *Land Degradation & Development* **27**, 14–25. doi:10.1002/ldr.2262

Paz-Ferreiro J, Gascó G, Gutiérrez B, Méndez A (2012) Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. *Biology and Fertility of Soils* **48**, 511–517. doi:10.1007/s00374-011-0644-3

Prasertsit K, Rattanawan N, Ratanapisit J (2011) Effects of wood vinegar as an additive for natural rubber products. *Songklanakarin Journal of Science and Technology* **33**, 425–430.

Rui Z, Wei D, Zhibin Y, Chao Z, Xiaojuan A (2014) Effects of wood vinegar on the soil microbial characteristics. *Journal of Chemical and Pharmaceutical Research* **6**, 1254–1260.

Schnurer J, Rosswall T (1982) Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and Environmental Microbiology* **43**, 1256–1261. doi:10.1128/AEM.43.6.1256-1261.1982

Schnurer J, Clarholm M, Rosswall T (1985) Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biology & Biochemistry* **17**, 611–618. doi:10.1016/0038-0717(85)90036-7

SISS [Societa' italiana Scienza del Suolo] (1995) 'Metodi Normalizzati di Analisi del Suolo.' (Edagricole: Bologna, Italy)

Souza JBG, Re-Poppia N, Raposo JL Jr (2012) Characterization of pyroligneous acid used in agriculture by gas chromatography-mass spectrometry. *Journal of the Brazilian Chemical Society* **23**, 610–617.

Steel RGD, Torrie JH, Dickey DA (1997) 'Principles and Procedures of Statistics.' (McGraw Hill: New York)

Steiner C, Teixeira WG, Lehmann J, Zech W (2004) Microbial response to charcoal amendments of highly weathered soils and Amazonian dark earths in central Amazonia-preliminary results. In 'Amazonian Dark Earths: Explorations in Space and Time'. (Ed. WI Woods) pp. 195–212. (Springer: Heidelberg)

Sun H, Feng Y, Xue L, Mandal S, Wang H, Shi W, Yang L (2020) Responses of ammonia volatilization from rice paddy soil to application of wood vinegar alone or combined with biochar. *Chemosphere* **242**, 125247. doi:10.1016/j.chemosphere.2019.125247

Tabatabai M (1994) Soil enzymes. Methods of soil analysis Part 2: microbiological and biochemical properties *SSSA Book Series 5.* **2**, 775–833.

Tabatabai MA, Bremner JM (1970) Arylsulfatase activity of soils. *Soil Science Society of America Proceedings* **34**, 225–229. doi:10.2136/sssaj1970.03615995003400020016x

Vance ED, Brookes C, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass carbon. *Soil Biology & Biochemistry* **19**, 703–707. doi:10.1016/0038-0717(87)90052-6

Zulkarami B, Ashrafuzzaman M, Husni MO, Ismail MR (2011) Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. *Australian Journal of Crop Science* **5**, 1508–1514.

Handling Editor: Leônidas Melo