Biodegradable plastics: Effects on functionality and fertility of two different soils

Published Version:
Mazzon, M., Gioacchini, P., Montecchio, D., Rapisarda, S., Ciavatta, C., Marzadori, C. (2022). Biodegradable plastics: Effects on functionality and fertility of two different soils. APPLIED SOIL ECOLOGY, 169, 1-11 [10.1016/j.apsoil.2021.104216].

Availability:
This version is available at: https://hdl.handle.net/11585/831443 since: 2021-09-07

Published:
DOI: http://doi.org/10.1016/j.apsoil.2021.104216

Biodegradable plastics: effects on functionality and fertility of two different soils

Martina Mazzon, Paola Gioacchini, Daniela Montecchio, Salvatore Rapisarda, Claudio Ciavatta, Claudio Marzadori*

Department of Agricultural and Food Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

*Corresponding author: Martina Mazzon, martina.mazzon2@unibo.it

ABSTRACT

In agriculture, the use of soil biodegradable mulch films could represent an eco-friendly alternative to conventional plastic films. However, soil biodegradable mulch films incorporated into the soil through tillage, being not only a physical but also a biogeochemical input, is expected to influence the soil quality by affecting its functions. Therefore, the eco-compatibility of these biodegradable plastics needs to be evaluated for their impact on different soil functions. To understand the effect of biodegradable plastics on soil quality (i.e. microbial biomass, nitrogen cycle, and activity of soil enzymes involved in the biochemical processes of carbon and nitrogen), we added increasing quantities of biodegradable plastics in two different soils: a loamy (Cambisol) and sandy (Arenosol) soil. The results highlight that the carbon added through the biodegradable plastics influenced the processes linked to carbon and nitrogen cycles. Significant effects were observed mainly with the highest dose of biodegradable plastics added (1%), resulting in a higher growth of microbial biomass, increased carbon mineralisation, and increased immobilisation of available nitrogen. The results also underline the importance of evaluating the impact of biodegradable plastics in different soils because all the processes considered are also influenced also by soil physicochemical characteristics.

Keywords

Biodegradable plastics; soil quality; soil respiration; soil microbial activity; soil enzyme activities

Highlights

- Biodegradable mulch films as eco-friendly alternative in agriculture
- Biodegradable mulch films incorporation could affect soil functionality
- Significant impact of biodegradable plastics on soil microbial biomass and activity
- Biodegradable plastics influenced the processes linked to soil C, N and P cycles
- Importance of considering soils with distinct characteristics

1. INTRODUCTION

Plastics are durable and cost-efficient materials that have been applied in a wide range of sectors, including agricultural production, particularly as plastic mulch (PlasticsEurope, 2018). In agriculture, plastic mulch contributes to increasing yields, extending the growing season, reducing weed pressure, improving fertiliser use efficiency, preserving soil moisture, and increasing soil temperature (Lalitha et al., 2010; Lamont, 2005). One of the major limitations to the use of plastic mulch is related to the operations and costs of removing and disposing of mulch film from the field at the end of the crop cycle; indeed incorrect removal and/or disposal of plastic mulch may cause environmental accumulation of fragmented materials and subsequent pollution of soil, water, and air resources (Moore-Kucera et al., 2014; Steinmetz et al., 2016).

The use of biodegradable plastics (BDP) as mulch films could represent an eco-friendly alternative to conventional plastic films. BDP mulch films offer the same agronomic advantages as plastic mulch films, but do not need to be removed and disposed of at the end of the crop cycle. Indeed, because of their biodegradability (according to the main standards, such as EN 17033:2018), they can be incorporated into the soil where they are used and mineralized by soil microorganisms, leading to reduced environmental impact and management costs (Brodhagen et al., 2015; Kyrikou and Briassoulis, 2007; Lucas et al., 2008). Once incorporated into the soil, BDP mulch films constitute a source of organic carbon (C), potentially influencing soil microbial biomass and activity. Consequently, these processes influence the biogeochemical cycles of elements and their bioavailability. Bandopadhyay et al. (2018) pointed out that the amount of C added to the soil for each single biodegradable plastic treatment is very small compared to the total volume of soil; however, it can cause an increase in microbial biomass and enzyme activity (Li et al., 2014; Yamamoto-Tamura et al., 2015). Several studies concerning the effects of conventional plastics on soil quality have been conducted taking into account physical, chemical, and biological parameters; however, conflicting conclusions have been reached. For example, Liu et al. (2017) studied the effect of polypropylene microplastics on the dynamics of soluble forms of C and nitrogen (N) (DOC, dissolved organic C and TDN, total dissolved N) and on soil enzyme activities. They found a stimulus of soil enzymatic activities that resulted in an increased availability of soluble C for microorganisms and nutrients (N and phosphorous (P)) for plants. In contrast, Awet et al. (2018) observed a reduction in soil dehydrogenase, N-(leucine-aminopeptidase), P-(alkaline–phosphatase) and C-(β-glucosidase and cellulose 1,4-beta-cellobiosidase) activities after the incorporation of polystyrene nanoparticles into the soil.

In the case of BDP, while biodegradation processes have been and are the subject of numerous studies in terms of mechanism and kinetics (Chinaglia et al., 2018; Dharmalingam et al., 2015; Hablot et al., 2014; Hayes et al., 2017; Kasirajan and Ngouajio, 2012; Kijchavengkul and Auras, 2008; Singh and Sharma, 2008; Tosin et al., 2019), only a few studies have investigated the effects of these materials on soil functionality, with results that are not always consistent (Bandopadhyay et al., 2018; Li et al., 2014; Qi et al., 2020; Sintim et al., 2019). This is mainly because of the presence of different edaphic factors (i.e. management systems, location, and season), as observed by Sintim et al. (2019). Generally, BDP have been shown to increase microbial biomass, respiration, enzyme activity, and fungal abundance (Li et al., 2014; Muroi et al., 2016); however, Moreno and Moreno (2008) found decreased microbial activity under mulching, and Moore-Kucera et al. (2014) found minimal effect of BDP on the microbial community. Bandopadhyay et al. (2018) highlighted the potential relationship between the microbial activity stimulated by BDP, microbial biomass, and soil organic matter (SOM) dynamics, whereas Li et al. (2014) found increased enzyme activity (β-glu) in soil with BDP and no corresponding increase in microbial biomass. Nonetheless, the effective eco-compatibility of these BDP needs to be evaluated by targeting the impact on different soil functions, particularly those related to the supply of nutrients and to the support of the microbial community. In this context, the amount and quality of C derived from BDP and their degradation by-products are expected to affect microbial growth and activity, as well as the composition of the microbial community. This, in turn, would influence the enzymatic activities directly involved in BDP degradation and/or in the release of nutrients needed for microbial growth and for the synthesis of the enzymes responsible for the degradation. Together, these mechanisms can affect the cycle of nutrients and their availability to plants; therefore, the soil functions are directly related to soil fertility (Bastida et al., 2008; Giacometti et al., 2013; Gil-Sotres et al., 2005; Mazzon et al., 2018; Trasar-Cepeda et al., 1998).

The objective of this study was to understand the effect of increasing amounts of BDP on soil functionality in two different soils that mainly differ in texture, with a high content of loam and clay in one, and a high content of sand in the other. Our aim was to determine which amount of BDP and derived C affect soil functionality measured by the use of chemical and biochemical parameters (growth and activity of the microbial biomass, N availability, and soil enzyme activities), which are fundamental in determining soil functionality.

2. MATERIALS AND METHODS

2.1 Experimental setup

A laboratory experiment was conducted over one year on two different agricultural soils: a loamy soil (Cambisol; WRB-IUSS, 2015) and a sandy soil (Arenosol; WRB-IUSS, 2015) (main characteristics are listed in Table 1). Both soils were collected from two farms in northern Italy (Piedmont Region). The two soils were cultivated without the use of BDP mulch films for horticultural production. The soils were sampled in September 2018, sieved at 2 mm, cleaned from plant debris, and stored at 4 °C. Two weeks before starting the experiment, the water content of the soils was adjusted to 60% of their water holding capacity (WHC) and kept at 23 °C. At the end of the pre-incubation period, 30 plastic containers (15 for each soil) with an equivalent of 700 g of dry soil were prepared. These corresponded to four biodegradable plastic treatments and one control (no plastic addition, CK), each carried out in triplicate.

The BDP used in this study are a commercial mulch film made of Mater-Bi (grade EF04P), a biodegradable plastic material produced by Novamont in the form of pellets, and certified "OK Biodegradable Soil" (TUV Austria). The mulch film is produced using Mater-Bi granules converted into film by film blowing with the addition of carbon black (approximately 2.8 %). Carbon black is supplemented using a masterbatch based on a biodegradable polymer present in Mater-Bi used in the production of the mulch film.

The amount of BDP added in the four treatments was 10 (P10), 100 (P100), 1,000 (P1000), and 10,000 (P10000) mg of biodegradable plastic per kg of dry soil (Table 2). The P100 treatment (100 mg/kg soil) corresponds to the mean annual quantity of BDP material incorporated into the soil (EN 17033 suggests 0.0063% = 63 mg/kg calculated based on mean characteristics of BDP), whereas the P10000 treatment corresponds to a loading rate of 1%, the quantity recommended in EN 17033. The BDP were added as small fragments (< 2 mm) and carefully mixed with the soil. The containers were covered with screw caps with a few holes to ensure gas exchange during incubation. The moisture content of each container was checked weekly and restored when necessary.

Sampling was carried out at 0, 28, 56, 112, 168, 224, and 350 days of incubation. At every sampling time, each container of soil was carefully mixed and the residual weight was recorded in order to maintain the same soil humidity throughout the experiment.

2.2 Soil respiration

Soil respiration was simultaneously measured for 35 days with a distinct incubation developed at 23 °C. For this analysis, moist soil samples, equivalent to 10 g of dry soil, were weighed in aluminium vessels, and the amount of BDP corresponding to the different treatments (Table 2) were added to the soil. The samples were then placed within airtight glass jars together with a glass vial containing 20 mL of 0.25 M NaOH. Twice a week, the vials were changed to new vials. Carbon dioxide (CO_2) released from the soil and trapped by NaOH was quantified using an elemental analyser for liquid samples (TOC-VCPH/CPN, Shimadzu Corp., Kyoto, Japan) and expressed as µg C-CO_2 g_{ds}^{-1} (Cheng, 2009).

2.3 Soil biochemical parameters

At every sampling time, microbial biomass C (MBC) and N (MBN) were measured using the fumigation-extraction method proposed by Vance et al. (1987). The C and N in the fumigated and non-fumigated extracts were determined using an elemental analyser for liquid samples (TOC-VCPH/CPN). The non-fumigated extracts were used to measure dissolved organic C (DOC) and total dissolved N (TDN). The C and N pools were expressed as mg kg_{ds}^{-1}.

Three enzymatic activities were measured during the experiment: dehydrogenase (Dehy) and β-glucosidase (β-glu) involved in the C cycle, and alkaline phosphatase (Phos) linked to P availability. The activity of these enzymes can be used as good indicators of soil quality and functionality (Gil-Sotres et al., 2005; Rao and Gianfreda, 2014). Dehy and β-glu are linked to the C cycle: the former (an intracellular enzyme) plays a marked role in the biological oxidation of SOM by transferring hydrogen from organic substrates to inorganic acceptors (Kumar et al., 2013), and the latter (an extracellular enzyme) hydrolases maltose, cellobiose and related products, which are important sources of energy for soil microorganisms (Ferraz De Almeida et al., 2015; Zhang et al., 2011). Both Dehy and β-glu are considered to be good soil quality indicators related to soil microbial activity (Dick and

Tabatabai, 1992; Gil-Sotres et al., 2005). Phos activity is related to the P cycle (P is the second-most limiting nutrient in agricultural production and a fundamental element for soil microbial activity) and is known to be a sensitive indicator of soil management changes (Acosta-Martínez and Tabatabai, 2011).

Dehy activity was determined according to the method described by von Mersi and Schinner (1991a). Moist soil (1g) was incubated with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 37 °C for 2 h. The release of 5-(4-iodophenyl)-1-(4-nitrophenyl)-3-phenylformazan (INTF) was measured at 464 nm and dehydrogenase activity was expressed as μg INTF g_{ds}^{-1} h^{-1}.

Phos activity was measured according to Eivazi and Tabatabai (1977) and β-glu was measured following Eivazi and Tabatabai (1988). For Phos, 1 g of moist soil was incubated with p-nitrophenyl-phosphate (pNP) as a substrate at 37 °C for 1 h. For β-glu, 1 g of soil was incubated with p-nitrophenyl-β-glucoside (pNG) at 37 °C for 1 h. The two enzymatic reactions release the same product, p-nitrophenol (pN), which is measured at 400 nm; therefore, Phos and β-glu activities are expressed as μg pN g_{ds}^{-1} h^{-1}.

Finally, the metabolic potential index (MI), an expression of soil metabolic activity related to the potential C sources for soil microbial metabolism and general microbial activity (Bastida et al., 2008), was obtained by dividing the Dehy activity by the dissolved organic C (Masciandaro et al., 2000, 1998). In general, the MI is used to assess variations in soil microbial activity after soil management changes, and decreases in MI indicate a reduction in microbial metabolic activity (Caravaca et al., 2002; Mazzon et al., 2018; Saviozzi et al., 2001).

2.4 Nitrification potential

The nitrification potential was determined following the procedure described by Berg and Rosswall (1985). This assay provides an index of the population size of autotrophic nitrifiers in the soil (Parker and Schimel, 2011). Briefly, 5 g of soil was incubated for 5 h in anoxic conditions with 20 mL of 1 mM ammonium sulphate as the substrate and 0.1 mL of sodium chlorate. The released nitrite was measured at 520 nm, and nitrification potential activity was expressed as ng N-NO_2^- g_{ds}^{-1} h^{-1}. The nitrification potential was measured at 112, 224, and 350 days of incubation.

2.5 Statistical analysis

Statistical analysis of the data was conducted using the R environment (R Core Team, 2020).

Soil cumulative respiration data were analysed by applying a negative exponential equation, the curve of which can be denoted as

$$CO_2 = CO_{2,max} \cdot (1 - e^{kt}) \quad (1)$$

where CO_2 is the quantity of CO_2 produced, t (day) is the time at which CO_2 concentration was measured, $CO_{2,max}$ is the asymptotic maximum quantity of CO_2 produced, and k is a parameter describing the shape of the curve (Creamer et al., 2014).

The effects of soil type, biodegradable plastic dose, and time were assessed using the function “anova_test” (rstatix package) for repeated measures ANOVA at a P level of 0.05. Previously, assumptions of normality, homogeneity, and sphericity were determined, and the Greenhouse-Geisser correction was used when needed. A pairwise t-test was then applied to determine the differences between soil type and biodegradable plastic dose within each measurement time ($P < 0.05$).

For C and N pools, MI, and the enzyme activities were also determined by soil type and biodegradable plastic dose effect over measurement time with a split-split plot ANOVA (P <

0.05) accounting for the repeated measures, followed by an LSD post hoc test ($P < 0.05$) with Bonferroni adjustment.

Finally, principal component analysis (PCA) was carried out using the “princomp” function. In order to assess if the separation between BDP doses was statistically significant, a PERMANOVA test was applied (“adonis” function with Euclidean distance).

3. RESULTS

3.1 Soil respiration

The CO_2 released during the first month of incubation, the calculated asymptotic maximum quantity of CO_2 produced, and the k parameter are listed in Table 3. The addition of BDP at lower doses (P10, P100, and P1000) induced different soil respiration (SR) responses in the two soils. In the loamy soil, SR was reduced compared to the control, whereas in the sandy soil, there was an increase in SR with dose, with 11%, 27%, and 40% SR for P10, P100, and P1000, respectively, although the difference was not statistically significant. In both soils, the only dose that caused a significant increase in SR compared to the control and other treatments was P10000 (Table 3). However, this increase was different between the two soils: in the loamy soil, only 49% more CO_2 was released compared to the control, whereas in the sandy soil, the extra CO_2 release was 435%.

In general, the loamy soil showed values of SR much closer to those of the calculated asymptotic maximum quantity of CO_2 produced ($CO_{2,max}$) compared to the sandy soil, where a greater discrepancy between the SR and the model was detected for all treatments. This aspect is clearly visible in the soil cumulative respiration graph (Figure 1), where it can be noted that, while the loamy soil was close to the plateau, the sandy soil was still rising and distant from the $CO_{2,max}$ value, thus indicating a greater oxidative capacity of sandy soil and a higher potential for CO_2 release.

3.2 Soil biochemical parameters

The changes in soil biochemical parameters over time during the incubation period are shown in Figures 2 and 3. The pattern of DOC over time showed a peak after two months of incubation in the loamy soil and then decreased to levels lower than the initial values. In the sandy soil, the observed fluctuations may represent cycles of immobilisation and release of DOC in the system, with values always higher than the initial values (Figure 2). Moreover, the sandy soil showed significantly higher values (+45% on average) of DOC than loamy soil for all measurement times. However, the behaviour of DOC was not influenced by BDP dose in either of the soils (Table 4). In contrast, the three other parameters displayed in Figure 2 (MBC, TDN, and MBN) were significantly affected by the highest dose of BDP and soil type (Table 4). MBC at day 0 was 50% higher in the loamy soil than in the sandy soil (Table 4). In both soils, after an initial decline in the first two months, MBC significantly increased in the P10000 treatment (from days 112 to 350). In the loamy soil, this increase in MBC was observed between days 56 and 168, and then the level decreased, although values at the end of the incubation period were still 70% higher than those of the other treatments. In the sandy soil, MBC increased with the P10000 treatment (+67%) until day 224 compared to the other treatments, and then tended to level off to the values of the control and other treatments. A similar pattern was also observed for MBN (Figure 2). Even for this parameter, from day 112 (Table 4), P10000 induced a significant increase in MBN (+68 and +48% compared to the other treatments in the sandy and loamy soils, respectively) with a pattern similar to that of MBC. The loamy soil showed significantly higher values (+62%) than the sandy soil on days 56 and 168 (Table 4).

A pattern opposite to that of MBC and MBN was observed for TDN that showed continuous N release during the entire experimental period for the control and lower doses of BDP

(Figure 2). Instead, consistent N immobilisation from day 56 was induced by P10000 in both soils, resulting in a decrease in TDN content with P10000, compared to the other treatments, with reduction reaching 200% between days 112 and 224.

In addition, the enzymatic activities and MI trend over time (Figure 3) showed fluctuations that were more accentuated in the loamy soil. Specifically, the main differences in Phos activity occurred from day 112 with loamy soil (Table 4), which showed higher values than the sandy soil (+59% on average) until the end of the experiment (day 350). In the loamy soil, P10000 treatment induced higher Phos activity than the other treatments at days 168 and 224 (+20%), whereas in the sandy soil, higher Phos activity occurred at days 112, 168, and 224 (+30%), and no significant differences between treatments were detected in both soils at day 350 (Table 4). β-glu, Dehy, and MI showed significant differences between soil type from days 0 to 350 (Table 4), with higher values in the loamy soil (β-glu +73%, Dehy +30%, and MI +73% on average) than in the sandy soil (Figure 3). When looking at the BDP effect (Figure 3), β-glu showed significantly higher values (+19%) with P10000 treatments in both soils only on day 112. Dehy showed significant differences mainly for the soil type - BDP interaction (Table 4); BDP effects were comparable between the two soils (Figure 3) only on days 168 and 224, with P10000 that was higher (+28%) compared to the other treatments. Finally, MI was affected by BDP only on days 56 and 168 (Table 4), but a clear increase (+25%) in both soils (Figure 3) with P10000 treatment was observed only on day 168.

Based on our findings, soil type affected all the considered parameters (Table S1), which showed higher values in the loamy soil with the exception of DOC, which was higher in the sandy soil, and TDN, which did not differ between the two soils (Figures 4 and 5). Within the BDP treatments, only P10000 (the highest dose) significantly differentiated from the other doses, but not for all the parameters considered. Indeed, P10000 treatment resulted in lower

TDN content and higher DOC, microbial biomass content (MBC and MBN), and Phos and Dehy activities.

PCA revealed that the first component (PC1) explained more than 30% and more than 50% of the total variance in the loamy and sandy soil, respectively, separating the P10000 treatment from the other treatments (Figure 6), as confirmed by the PERMANOVA test (Table 5). Moreover, from the PCA, it seems that microbial biomass (MBC and MBN) and Dehy activity were the most characteristic parameters of the P10000 treatment in both soils.

3.3 Nitrification potential

Changes in nitrification potential over time (Table S2) during the incubation period are shown in Figure 7. In general, loamy soil showed a higher nitrification potential than sandy soil (Figure 7, Table 6). The P10000 BDP dose increased the nitrification potential by 26%, compared to the other treatments, in the loamy soil during the whole incubation period, whereas in the sandy soil, an increase of 29% was detected only at the first sampling time, corresponding to day 112 of incubation (Figure 7). Afterwards, in the sandy soil, the nitrification potential significantly decreased and levelled off in all treatments.

4. DISCUSSION

4.1 Soil respiration

During the first month of incubation, only the highest dose of BDP (1%) caused an increase in CO_2 losses compared to the other treatments and the control. However, this stimulus was greater in the sandy soil than in the loamy soil. In our experiment, we were not able to distinguish the source of this extra CO_2 released from the soil after the addition of BDP. Therefore, not only the degradation of BDP by soil microorganisms could have accounted for this increased loss of CO_2, but also the mechanisms related to the priming effect. Indeed, the addition of C substrate to soil, such as the that applied in our study, actually impacts microbial activity and can cause either an acceleration of microbial biomass turnover (apparent priming effect) or a change in the mineralisation of the SOM as a result of a real priming effect (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000). Real priming is usually observed with complex substrates poor in N, where microorganisms use native SOM to recover energy and N for the synthesis of enzymes capable of metabolising the substrate. Therefore, the N limitation induced by a surplus of complex C added to the soil stimulates N mining from native SOM, which is one of the mechanisms responsible for the real priming effect (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014). In our experiment, treatment with the highest dose of BDP supplied a large amount of C (6 g kg_{ds}^{-1}) but not N, and in this context, a real priming effect might have contributed to the increased SR. This increase, however, was greater in the sandy soil, where the calculated $CO_{2,max}$ value was two times higher than that of the loamy soil, supporting that the former has a higher C mineralisation capacity over time than the latter. The lower release of CO_2 from the loamy soil after BDP addition was also evident with the lower doses that caused a reduction in the CO_2 released from soil compared to the control. Therefore, not only the amount of BDP and the related C added to the soil is an important factor in terms of stimuli to SR, but soil characteristics also play an important role in the regulation of this process (Sintim et al., 2019).

4.2 Soil biochemical parameters

In both soils at the end of the first month of incubation, most of the C added with the BDP still remained in the system, potentially available to the soil microbial community. However, only the highest BDP treatment supplied a dose of C able to stimulate a significant response in terms of microbial biomass content, Dehy and Phos activities, and N immobilisation. The increase in C availability with the P10000 treatment may have stimulated microbial growth and activity and caused a decrease in TDN content (Sinsabaugh, 2010), which has become the limiting element in this context (Li et al., 2014). The same result was observed by Li et al. (2014), who attributed the N dynamic to the fact that biodegradable films (such as the ones used in the present study) are generally C-rich but nutrient-poor. In particular, the BDP tested in our experiment did not contain any N and, unlike the lower doses that did not affect the C/N ratio of the soil, the highest dose caused a shift in soil C/N ratio from 10 to 14 in the loamy soil and from 9 to 13 in the sandy soil (Table 2). Therefore, the demand for N by the microbial biomass that we observed in our study might be driven either by this consistent shift in C/N ratio of the soil towards higher values, or by the structural complexity of this kind of material (Sinsabaugh, 2010). These two aspects affect the dynamics of microbial biomass, N immobilisation, and enzymatic activities. According to the stoichiometric decomposition theory, the C/N ratio of the substrate, compared to that of the microbial biomass, drives the decomposition process and regulates the amount of C and N that is used for microbial growth, which is released as CO_2 and mineral N (Barrett and Burke, 2000; Hessen et al., 2004; Mooshammer et al., 2014b). The addition of the highest dose of BDP, which is a C-rich substrate without N, increased the C content of the system but also caused a stoichiometric imbalance between the substrate and microbial biomass, thereby leading to the sequestration of soil available N by soil microorganisms. The demand for N during the incubation period may have also stimulated the decomposition of SOM for N mining by the microbial biomass (Moorhead and Sinsabaugh, 2006). Moreover, BDP are a complex substrate that need to be deconstructed through the activity of extracellular enzymes to make its C available to the microbial biomass (Caldwell, 2005). The production of extracellular enzymes also requires N and can further contribute to N sequestration (Mooshammer et al., 2014a; Schimel and Weintraub, 2003). In our study, we observed in both soils a clear and significant effect of the BDP treatment on the N dynamics only after two months of incubation, when N immobilisation clearly started in both soils in the presence of the highest BDP dose (Table 4, Figure 2). This caused significant microbial growth in both soils from day 112, which remained high until day 224. At approximately the same time range (days 112–224), the highest levels of dehydrogenase and phosphatase activities were observed. The increased Dehy activity could be related to the SOM degradation (N mining) induced by the demand for N (Kumar et al., 2013; Piotrowska-Długosz, 2014; Srinivasulu and Rangaswamy, 2014). Moreover, Phos activity could be related to the demand for P by the growing microbial biomass, given that both N and P could become limiting nutrients for microbial growth in the presence of a C-rich substrate (Mooshammer et al., 2014). However, a net N release was observed with P10000 treatment in both soils with TDN, which increased by 56% and 59% from days 224 to 350 in loamy and sandy soil, respectively. During the same time interval, with P10000 treatment, the microbial biomass decreased by 32%–45%, and the same trend was observed for the abovementioned enzymatic activities. Together, these results could indicate a strong microbial turnover confirmed by the reduction of Dehy activity, which is only present in active microorganisms, and therefore, could reflect the death of some of the soil microorganisms and their subsequent turnover (Bello et al., 2014). The trend over time observed for microbial biomass, TDN, and Dehy and Phos activities was not observed for β-glu activity and MI. Contrary to our results, Li et al. (2014) observed an increase in β-glu activity without a corresponding increase in microbial biomass, suggesting that there was a more efficient metabolic process for the microbial community and that β-glu activity is a responsive parameter for testing mulch effects on soil. In our study, we did not observe any marked increase in β-glu activity with P10000 treatment. This could be attributed to the complexity of BDP as substrates, which may reach a degree of degradation that is not sufficient to bring biodegradation products suitable for β-glu utilisation to the soil. Indeed, it is known that β-glu is mainly involved in the last stage of degradation of C-substrates as glucosidases hydrolyse the degradation products of amylase and cellulose (Deng and Popova, 2011; Piotrowska-Długosz, 2014). The soil type significantly impacted all enzymatic activities and MI (Table 4, Figure 3), with higher values in loamy soil. This was not a surprising result, as it is known that enzymes are strongly adsorbed by clays, which influence their activity and stability in the soil (Burns, 1982, 1978; Monreal and Bergstrom, 2000; Saviozzi et al., 1997).

However, considering all these parameters (microbial biomass, MI, enzyme activities, and available C and N) together in the PCA analysis (Figure 6) for both soils revealed a strong relationship of P10000 treatment with microbial biomass (MBC and MBN) and activity (Dehy activity), confirming that BDP addition strongly affected the microbial community (Bandopadhyay et al., 2018) potentially by stimulating SOM degradation and N mining.

4.3 Nitrification potential

The nitrification potential, similar to most of the observed parameters, was much higher in the loamy soil than in the sandy soil. The lower doses of BDP always showed similar results to the control, and did not significantly affect the nitrification process. Our results with the lower doses confirm the findings of Bettas Ardisson et al. (2014), who did not observe any negative effect on the nitrification rate after tillage with BDP mulches.

A significant increase in the nitrification potential was observed only with the highest dose of BDP in loamy soil at all sampling times. In sandy soil, this effect was observed only at the first sampling time (day 112 of incubation) with P10000, which was 29% higher than that of the other treatments; afterwards, the values became similar to the control.

As previously observed, the highest BDP dose stimulated a general increase in microbial biomass and a greater N immobilisation as a result of the higher amount of C added. High levels of MBN measured in soils receiving high C inputs were reported to be accompanied by high rates of gross ammonification due to the high N demand (Burger and Jackson, 2003). As already hypothesised, in our study, the elevated and prolonged N demand during the period of maximal microbial growth (days 56–224) may have stimulated the mineralisation of SOM and the ammonification rate. In this phase, however, the N released from SOM mineralisation may be simultaneously immobilised by the heterotrophic microbial community that preferentially assimilates mineral N in the NH_4^+ form (Recous et al., 1990; Rice and Tiedje, 1989; Shi et al., 2004; Shi and Norton, 2000) and is more competitive for NH_4^+ compared to nitrifiers (Johnson, 1992; Recous et al., 1990; Schimel et al., 1989). Consequently, despite the higher nitrification potential, the level of TDN remained significantly lower than the other treatments. Moreover, we cannot exclude the possibility that some NO_3^- may also be immobilised. Indeed, in the presence of a high amount of complex C substrate, NO_3^- has been found to be immobilised to a certain extent (Burger and Jackson, 2003; Cheng et al., 2017).

5. CONCLUSIONS

The addition of C from BDP influenced the processes linked to the C and N cycles, with positive effects on soil microbial biomass, even if the extent of the processes was significantly influenced by the physicochemical characteristics of the soils considered. Indeed, C and N dynamics and enzyme activities were strongly affected by soil texture, independent of the BDP dose added.

The lower doses of BDP (P10, P100, and P1000) induced results that were comparable to those of the control, indicating that their addition to the soil did not affect the soil biochemistry. Only the highest dose of BDP (P10000) stimulated growth of the microbial biomass, increased C mineralisation, and increased immobilisation of available N. Indeed, addition of C with the highest BDP dose caused an imbalance in the C/N ratio, thereby increasing the need for microorganisms to immobilise N, the limiting element. This, in turn, stimulated the microbial activity for SOM decomposition and N mining (priming effect).

Our study has clearly shown that, independent of soil physical-chemical characteristics, BDP addition at higher dose (1%) induces an imbalance in C/N stoichiometry, which opens the road for future investigation that should include plant and N supply in order to evaluate BDP effects on both soil and plants.

CRediT authorship contribution statement

Mazzon: methodology, formal analysis, writing, review and editing

Gioacchini: conceptualization, investigation, writing, review and editing

Montecchio: conceptualization, investigation, review and editing

Rapisarda: investigation, methodology, writing

Ciavatta: conceptualization, supervision, funding acquisition

Marzadori: conceptualization, supervision, funding acquisition

Funding sources

This research was supported by Novamont S.p.A..

References

Acosta-Martínez, V., Tabatabai, M.A., n.d. Phosphorus cycle enzymes, in: Dick, R.P. (Ed.), Methods of Soil Enzymology. SSSA Book series 9.

Awet, T.T., Kohl, Y., Meier, F., Straskraba, S., Grün, A.L., Ruf, T., Jost, C., Drexel, R., Tunc, E., Emmerling, C., 2018. Effects of polystyrene nanoparticles on the microbiota and functional diversity of enzymes in soil. Environ. Sci. Eur. 30. https://doi.org/10.1186/s12302-018-0140-6

Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., 2018. Biodegradable plastic mulch films: Impacts on soil microbial communities and ecosystem functions. Front. Microbiol. 9, 1–7. https://doi.org/10.3389/fmicb.2018.00819

Barrett, J.E., Burke, I.C., 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. Soil Biol. Biochem. 32, 1707–1716. https://doi.org/10.1016/S0038-0717(00)00089-4

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices: A biological perspective. Geoderma 147, 159–171. https://doi.org/10.1016/j.geoderma.2008.08.007

Bello, D., Trasar-Cepeda, C., Gil-Sotres, F., 2014. Enzymes and Environmental Contaminants Significant to Agricultural Sciences, in: Enzymes in Agricultural Sciences; Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.

Berg, P., Rosswall, T., 1985. Ammonium oxidizer numbers, potential and actual oxidation rates in two swedish arable soils. Biol. Fertil. Soils 1, 131–140. https://doi.org/10.1007/BF00301780

Bettas Ardisson, G., Tosin, M., Barbale, M., Degli-Innocenti, F., 2014. Biodegradation of plastics in soil and effects on nitrification activity. A laboratory approach. Front. Microbiol. 5, 1–7. https://doi.org/10.3389/fmicb.2014.00710

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biol. Fertil. Soils 45, 115–131. https://doi.org/10.1007/s00374-008-0334-y

Brodhagen, M., Peyron, M., Miles, C., Inglis, D.A., 2015. Biodegradable plastic agricultural mulches and key features of microbial degradation. Appl. Microbiol. Biotechnol. 99, 1039–1056. https://doi.org/10.1007/s00253-014-6267-5

Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol. Biochem. 35, 29–36. https://doi.org/10.1016/S0038-0717(02)00233-X

Burns, R.G., 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol. Biochem. 14, 423–427. https://doi.org/10.1016/0038-0717(82)90099-2

Burns, R.G., 1978. Soil enzymes. Academic Press.

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia (Jena). 49, 637–644. https://doi.org/10.1016/j.pedobi.2005.06.003

Caravaca, F., Masciandaro, G., Ceccanti, B., 2002. Land use in relation to soil chemical and biochemical properties in a semiarid Mediterranean environment. Soil Tillage Res. 68, 23–30. https://doi.org/10.1016/S0167-1987(02)00080-6

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 20. https://doi.org/10.1111/gcb.12475

Cheng, J., Chen, Y., He, T., Liao, R., Liu, R., Yi, M., Huang, L., Yang, Z., Fu, T., Li, X., 2017. Soil nitrogen leaching decreases as biogas slurry DOC/N ratio increases. Appl. Soil Ecol. 111, 105–113. https://doi.org/10.1016/j.apsoil.2016.12.001

Cheng, W., 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biol. Biochem. 41, 1795–1801. https://doi.org/10.1016/j.soilbio.2008.04.018

Chinaglia, S., Tosin, M., Degli-Innocenti, F., 2018. Biodegradation rate of biodegradable plastics at molecular level. Polym. Degrad. Stab. 147, 237–244. https://doi.org/10.1016/j.polymdegradstab.2017.12.011

Creamer, R.E., Schulte, R.P.O., Stone, D., Gal, A., Krogh, P.H., Lo Papa, G., Murray, P.J., Pérès, G., Foerster, B., Rutgers, M., Sousa, J.P., Winding, A., 2014. Measuring basal soil respiration across Europe: Do incubation temperature and incubation period matter? Ecol. Indic. 36, 409–418. https://doi.org/10.1016/j.ecolind.2013.08.015

Deng, S., Popova, I., 2011. Carbohydrate hydrolases., in: Dick, R.P. (Ed.), Methods of Soil Enzymology. SSSA Book series 9.

Dharmalingam, S., Hayes, D.G., Wadsworth, L.C., Dunlap, R.N., DeBruyn, J.M., Lee, J., Wszelaki, A.L., 2015. Soil Degradation of Polylactic Acid/Polyhydroxyalkanoate-Based Nonwoven Mulches. J. Polym. Environ. 23, 302–315. https://doi.org/10.1007/s10924-015-0716-9

Dick, W.A., Tabatabai, M.A., 1992. Significance and potential uses of soil enzymes, in: Metting, F.B. (Ed.), Soil Microbial Ecology - Applications in Agricultural and Environmental Management. Mercel Dekker, Inc.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Eivazi, F., Tabatabai, M.A., 1977. Phosphates in soils. Soil Biol. Biochem. 9, 167–172.

Ferraz De Almeida, R., Naves, E.R., Pinheiro, R., Mota, D., 2015. Soil quality: Enzymatic activity of soil β-glucosidase. Glob. J. Agric. Res. Rev. 3, 2437–1858.

Giacometti, C., Demyan, M.S., Cavani, L., Marzadori, C., Ciavatta, C., Kandeler, E., 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. Appl. Soil Ecol. 64, 32–48.

https://doi.org/10.1016/j.apsoil.2012.10.002

Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37, 877–887. https://doi.org/10.1016/j.soilbio.2004.10.003

Hablot, E., Dharmalingam, S., Hayes, D.G., Wadsworth, L.C., Blazy, C., Narayan, R., 2014. Effect of Simulated Weathering on Physicochemical Properties and Inherent Biodegradation of PLA/PHA Nonwoven Mulches. J. Polym. Environ. 22, 417–429. https://doi.org/10.1007/s10924-014-0697-0

Hayes, D.G., Wadsworth, L.C., Sintim, H.Y., Flury, M., English, M., Schaeffer, S., Saxton, A.M., 2017. Effect of diverse weathering conditions on the physicochemical properties of biodegradable plastic mulches. Polym. Test. 62, 454–467. https://doi.org/10.1016/j.polymertesting.2017.07.027

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon sequestration in ecosystems: The role of stoichiometry. Ecology 85, 1179–1192. https://doi.org/10.1890/02-0251

Johnson, D.W., 1992. Nitrogen Retention in Forest Soils. J. Environ. Qual. 21, 1–12. https://doi.org/10.1001/jama.1963.03700080014004

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and biodegradable mulches for agricultural applications: A review. Agron. Sustain. Dev. 32, 501–529. https://doi.org/10.1007/s13593-011-0068-3

Kijchavengkul, T., Auras, R., 2008. Compostability of polymers. Polym Int 57, 793:804. https://doi.org/10.1002/pi.2420

Kumar, S., Chaudhuri, S., Maiti, S.K., 2013. Soil dehydrogenase enzyme activity in natural and mine soil - A review. Middle East J. Sci. Res. 13, 898–906. https://doi.org/10.5829/idosi.mejsr.2013.13.7.2801

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Kyrikou, I., Briassoulis, D., 2007. Biodegradation of agricultural plastic films: A critical review. J. Polym. Environ. 15, 125–150. https://doi.org/10.1007/s10924-007-0053-8

Lalitha, M., Thilagam, V., Balakrishnan, N., Mansour, M., 2010. Effect of Plastic Mulch on Soil Properties and Crop Growth - A Review. Agric. Rev. 31, 145–149.

Lamont, W.J., 2005. Plastics: Modifying the microclimate for the production of vegetable crops. Horttechnology 15, 477–481. https://doi.org/10.21273/horttech.15.3.0477

Li, C., Moore-Kucera, J., Lee, J., Corbin, A., Brodhagen, M., Miles, C., Inglis, D., 2014. Effects of biodegradable mulch on soil quality. Appl. Soil Ecol. 79, 59–69. https://doi.org/10.1016/j.apsoil.2014.02.012

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., Ritsema, C.J., Geissen, V., 2017. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185, 907–917. https://doi.org/10.1016/j.chemosphere.2017.07.064

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.E., 2008. Polymer biodegradation: Mechanisms and estimation techniques - A review. Chemosphere 73, 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064

Masciandaro, G., Ceccanti, B., Gallardo-Lancho, J.F., 1998. Organic matter properties in cultivated versus set-aside arable soils. Agric. Ecosyst. Environ. 67, 267–274. https://doi.org/10.1016/S0167-8809(97)00124-2

Masciandaro, G., Ceccanti, B., Ronchi, V., Bauer, C., 2000. Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilisers. Biol. Fertil. Soils 32, 479–483. https://doi.org/10.1007/s003740000280

Mazzon, M., Cavani, L., Margon, A., Sorrenti, G., Ciavatta, C., Marzadori, C., 2018. Changes in soil phenol oxidase activities due to long-term application of compost and mineral N in a walnut orchard. Geoderma 316. https://doi.org/10.1016/j.geoderma.2017.12.009

Monreal, C.M., Bergstrom, D.W., 2000. Soil enzymatic factors expressing the influence of land use, tillage system and texture on soil biochemical quality. Can. J. Soil Sci. 80, 419–428. https://doi.org/10.4141/S99-088

Moore-Kucera, J., Cox, S.B., Peyron, M., Bailes, G., Kinloch, K., Karich, K., Miles, C., Inglis, D.A., Brodhagen, M., 2014. Native soil fungi associated with compostable plastics in three contrasting agricultural settings. Appl. Microbiol. Biotechnol. 98, 6467–6485. https://doi.org/10.1007/s00253-014-5711-x

Moorhead, Daryl L. and Sinsabaugh, R.L., 2006. Mosaic Patterns of Thermal Stress in the Rocky Intertidal Zone: Implications for Climate Change. Ecol. Monogr. 76, 151–174. https://doi.org/10.1890/0012-9615(2006)076

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014a. Adjustment of microbial nitrogen use efficiency to carbon:Nitrogen imbalances regulates soil nitrogen cycling. Nat. Commun. 5, 1–7. https://doi.org/10.1038/ncomms4694

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014b. Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 5, 1–10. https://doi.org/10.3389/fmicb.2014.00022

Moreno, M.M., Moreno, A., 2008. Effect of different biodegradable and polyethylene mulches on soil properties and production in a tomato crop. Sci. Hortic. (Amsterdam). 116, 256–263. https://doi.org/10.1016/j.scienta.2008.01.007

Muroi, F., Tachibana, Y., Kobayashi, Y., Sakurai, T., Kasuya, K.I., 2016. Influences of poly(butylene adipate-co-terephthalate) on soil microbiota and plant growth. Polym. Degrad. Stab. 129, 338–346. https://doi.org/10.1016/j.polymdegradstab.2016.05.018

Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Appl. Soil Ecol. 48, 185–192. https://doi.org/10.1016/j.apsoil.2011.03.007

Piotrowska-Długosz, A., 2014. Enzymes and soil fertility, in: Enzymes in Agricultural Sciences; Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.

PlasticsEurope, 2018. Plastics – the Facts 2017. An analysis of European plastics production, demand and waste data. www.plasticseurope.org 38.

Qi, Y., Beriot, N., Gort, G., Huerta Lwanga, E., Gooren, H., Yang, X., Geissen, V., 2020. Impact of plastic mulch film debris on soil physicochemical and hydrological properties. Environ. Pollut. 266, 115097. https://doi.org/10.1016/j.envpol.2020.115097

R Core Team, 2020. R: A language and environment for statistical computing.

Rao, M.A., Gianfreda, L., 2014. Soil enzymes, in: Enzymes in Agricultural Sciences; Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.

Recous, S., Mary, B., Faurie, G., 1990. Microbial immobilization of ammonium and nitrate in cultivated soils. Soil Biol. Biochem. 22, 913–922. https://doi.org/10.1016/0038-

0717(90)90129-N

Rice, C.W., Tiedje, J.M., 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. Soil Biol. Biochem. 21, 597–602. https://doi.org/10.1016/0038-0717(89)90135-1

Saviozzi, A., Levi-Minzi, R., Cardelli, R., Riffaldi, R., 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant Soil 233, 251–259. https://doi.org/10.1023/A:1010526209076

Saviozzi, A., Riffaldi, R., Levi-Minzi, R., Panichi, A., 1997. Properties of soil particle size separates after 40 years of continuous corn. Commun. Soil Sci. Plant Anal. 28, 427–440. https://doi.org/10.1080/00103629709369801

Schimel, J.P., Jackson, L.E., Firestone, M.K., 1989. SPATIAL AND TEMPORAL EFFECTS ON PLANT-MICROBIAL COMPETITION FOR INORGANIC NITROGEN IN A CALIFORNIA ANNUAL GRASSLAND. Soil Biol. Biochem. 21, 1059–1066. https://doi.org/10.1016/S0367-326X(99)00140-9

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. Soil Biol. Biochem. 35, 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Shi, W., Miller, B.E., Stark, J.M., Norton, J.M., 2004. Microbial Nitrogen Transformations in Response to Treated Dairy Waste in Agricultural Soils. Soil Sci. Soc. Am. J. 68, 1867–1874. https://doi.org/10.2136/sssaj2004.1867

Shi, W., Norton, J.M., 2000. Microbial control of nitrate concentrations in an agricultural soil treated with dairy waste compost or ammonium fertilizer. Soil Biol. Biochem. 32, 1453–1457. https://doi.org/10.1016/S0038-0717(00)00050-X

Singh, B., Sharma, N., 2008. Mechanistic implications of plastic degradation. Polym. Degrad. Stab. 93, 561–584. https://doi.org/10.1016/j.polymdegradstab.2007.11.008

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sintim, H.Y., Bandopadhyay, S., English, M.E., Bary, A.I., DeBruyn, J.M., Schaeffer, S.M., Miles, C.A., Reganold, J.P., Flury, M., 2019. Impacts of biodegradable plastic mulches on soil health. Agric. Ecosyst. Environ. 273, 36–49. https://doi.org/10.1016/j.agee.2018.12.002

Srinivasulu, M., Rangaswamy, V., 2014. Enzymes and pesticides, in: Enzymes in Agricultural Sciences; Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Schaumann, G.E., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550, 690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Tosin, M., Pischedda, A., Degli-Innocenti, F., 2019. Biodegradation kinetics in soil of a multi-constituent biodegradable plastic. Polym. Degrad. Stab. 166, 213–218. https://doi.org/10.1016/j.polymdegradstab.2019.05.034

Trasar-Cepeda, C., Leirós, C., Gil-Sotres, F., Seoane, S., 1998. Towards a biochemical quality index for soils: An expression relating several biological and biochemical properties. Biol. Fertil. Soils 26, 100–106. https://doi.org/10.1007/s003740050350

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Eiol. Biochem 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6 von Mersi, W., Schinner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol. Fertil. Soils 11, 216–220. https://doi.org/10.1007/BF00335770

WRB-IUSS, 2015. World Reference Base for Soil Resources. World Soil Resources Reports 106, World Soil Resources Reports No. 106. Rome.

Yamamoto-Tamura, K., Hiradate, S., Watanabe, T., Koitabashi, M., Sameshima-Yamashita, Y., Yarimizu, T., Kitamoto, H., 2015. Contribution of soil esterase to biodegradation of aliphatic polyester agricultural mulch film in cultivated soils. AMB Express 5. https://doi.org/10.1186/s13568-014-0088-x

Zhang, Y., Chen, L., Wu, Z., Sun, C., 2011. Kinetic parameters of soil β-glucosidase response to environmental temperature and moisture regimes. Rev. Bras. Ciência do Solo 35, 1285–1291. https://doi.org/10.1590/s0100-06832011000400022