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

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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

86 targeting the impact on different soil functions, particularly those related to the supply of
87 nutrients and to the support of the microbial community. In this context, the amount and
88 quality of C derived from BDP and their degradation by-products are expected to affect
89 microbial growth and activity, as well as the composition of the microbial community. This,
90 in turn, would influence the enzymatic activities directly involved in BDP degradation and/or
91 in the release of nutrients needed for microbial growth and for the synthesis of the enzymes
92 responsible for the degradation. Together, these mechanisms can affect the cycle of
93 nutrients and their availability to plants; therefore, the soil functions are directly related to
94 soil fertility (Bastida et al., 2008; Giacometti et al., 2013; Gil-Sotres et al., 2005; Mazzon et
95 al., 2018; Trasar-Cepeda et al., 1998).
96 The objective of this study was to understand the effect of increasing amounts of BDP on
97 soil functionality in two different soils that mainly differ in texture, with a high content of loam
98 and clay in one, and a high content of sand in the other. Our aim was to determine which
99 amount of BDP and derived C affect soil functionality measured by the use of chemical and
100 biochemical parameters (growth and activity of the microbial biomass, N availability, and soil
101 enzyme activities), which are fundamental in determining soil functionality.
102

103 ## 2. MATERIALS AND METHODS

104 *2.1 Experimental setup*

105 A laboratory experiment was conducted over one year on two different agricultural soils: a
106 loamy soil (Cambisol; WRB-IUSS, 2015) and a sandy soil (Arenosol; WRB-IUSS, 2015)
107 (main characteristics are listed in Table 1). Both soils were collected from two farms in
108 northern Italy (Piedmont Region). The two soils were cultivated without the use of BDP
109 mulch films for horticultural production. The soils were sampled in September 2018, sieved
110 at 2 mm, cleaned from plant debris, and stored at 4 °C. Two weeks before starting the

111 experiment, the water content of the soils was adjusted to 60% of their water holding
112 capacity (WHC) and kept at 23 °C. At the end of the pre-incubation period, 30 plastic
113 containers (15 for each soil) with an equivalent of 700 g of dry soil were prepared. These
114 corresponded to four biodegradable plastic treatments and one control (no plastic addition,
115 CK), each carried out in triplicate.
116 The BDP used in this study are a commercial mulch film made of Mater-Bi (grade EF04P),
117 a biodegradable plastic material produced by Novamont in the form of pellets, and certified
118 “OK Biodegradable Soil” (TUV Austria). The mulch film is produced using Mater-Bi granules
119 converted into film by film blowing with the addition of carbon black (approximately 2.8 %).
120 Carbon black is supplemented using a masterbatch based on a biodegradable polymer
121 present in Mater-Bi used in the production of the mulch film.
122 The amount of BDP added in the four treatments was 10 (P10), 100 (P100), 1,000 (P1000),
123 and 10,000 (P10000) mg of biodegradable plastic per kg of dry soil (Table 2). The P100
124 treatment (100 mg/kg soil) corresponds to the mean annual quantity of BDP material
125 incorporated into the soil (EN 17033 suggests 0.0063% = 63 mg/kg calculated based on
126 mean characteristics of BDP), whereas the P10000 treatment corresponds to a loading rate
127 of 1%, the quantity recommended in EN 17033. The BDP were added as small fragments
128 (< 2 mm) and carefully mixed with the soil. The containers were covered with screw caps
129 with a few holes to ensure gas exchange during incubation. The moisture content of each
130 container was checked weekly and restored when necessary.
131 Sampling was carried out at 0, 28, 56, 112, 168, 224, and 350 days of incubation. At every
132 sampling time, each container of soil was carefully mixed and the residual weight was
133 recorded in order to maintain the same soil humidity throughout the experiment.
134
135 *2.2 Soil respiration*

136 Soil respiration was simultaneously measured for 35 days with a distinct incubation
137 developed at 23 °C. For this analysis, moist soil samples, equivalent to 10 g of dry soil, were
138 weighed in aluminium vessels, and the amount of BDP corresponding to the different
139 treatments (Table 2) were added to the soil. The samples were then placed within airtight
140 glass jars together with a glass vial containing 20 mL of 0.25 M NaOH. Twice a week, the
141 vials were changed to new vials. Carbon dioxide (CO_2) released from the soil and trapped
142 by NaOH was quantified using an elemental analyser for liquid samples (TOC-VCPH/CPN,
143 Shimadzu Corp., Kyoto, Japan) and expressed as μg C-CO_2 g_{ds}^{-1} (Cheng, 2009).
144
145 *2.3 Soil biochemical parameters*

146 At every sampling time, microbial biomass C (MBC) and N (MBN) were measured using the
147 fumigation-extraction method proposed by Vance et al. (1987). The C and N in the fumigated
148 and non-fumigated extracts were determined using an elemental analyser for liquid samples
149 (TOC-VCPH/CPN). The non-fumigated extracts were used to measure dissolved organic C
150 (DOC) and total dissolved N (TDN). The C and N pools were expressed as mg kg_{ds}^{-1}.
151 Three enzymatic activities were measured during the experiment: dehydrogenase (Dehy)
152 and β-glucosidase (β-glu) involved in the C cycle, and alkaline phosphatase (Phos) linked
153 to P availability. The activity of these enzymes can be used as good indicators of soil quality
154 and functionality (Gil-Sotres et al., 2005; Rao and Gianfreda, 2014). Dehy and β-glu are
155 linked to the C cycle: the former (an intracellular enzyme) plays a marked role in the
156 biological oxidation of SOM by transferring hydrogen from organic substrates to inorganic
157 acceptors (Kumar et al., 2013), and the latter (an extracellular enzyme) hydrolases maltose,
158 cellobiose and related products, which are important sources of energy for soil
159 microorganisms (Ferraz De Almeida et al., 2015; Zhang et al., 2011). Both Dehy and β-glu
160 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

185 The nitrification potential was determined following the procedure described by Berg and
186 Rosswall (1985). This assay provides an index of the population size of autotrophic nitrifiers
187 in the soil (Parker and Schimel, 2011). Briefly, 5 g of soil was incubated for 5 h in anoxic
188 conditions with 20 mL of 1 mM ammonium sulphate as the substrate and 0.1 mL of sodium
189 chlorate. The released nitrite was measured at 520 nm, and nitrification potential activity
190 was expressed as ng $N\text{-}NO_2^-$ g_{ds}^{-1} h^{-1}. The nitrification potential was measured at 112, 224,
191 and 350 days of incubation.
192
193 *2.5 Statistical analysis*
194 Statistical analysis of the data was conducted using the R environment (R Core Team,
195 2020).
196 Soil cumulative respiration data were analysed by applying a negative exponential equation,
197 the curve of which can be denoted as

198 $$CO_2 = CO_{2,max} \cdot (1 - e^{kt}) \quad (1)$$

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

202 The effects of soil type, biodegradable plastic dose, and time were assessed using the
203 function “anova_test” (rstatix package) for repeated measures ANOVA at a P level of 0.05.
204 Previously, assumptions of normality, homogeneity, and sphericity were determined, and
205 the Greenhouse-Geisser correction was used when needed. A pairwise t-test was then
206 applied to determine the differences between soil type and biodegradable plastic dose within
207 each measurement time ($P < 0.05$).
208 For C and N pools, MI, and the enzyme activities were also determined by soil type and
209 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.

235

236 *3.2 Soil biochemical parameters*

237 The changes in soil biochemical parameters over time during the incubation period are
238 shown in Figures 2 and 3. The pattern of DOC over time showed a peak after two months
239 of incubation in the loamy soil and then decreased to levels lower than the initial values. In
240 the sandy soil, the observed fluctuations may represent cycles of immobilisation and release
241 of DOC in the system, with values always higher than the initial values (Figure 2). Moreover,
242 the sandy soil showed significantly higher values (+45% on average) of DOC than loamy
243 soil for all measurement times. However, the behaviour of DOC was not influenced by BDP
244 dose in either of the soils (Table 4). In contrast, the three other parameters displayed in
245 Figure 2 (MBC, TDN, and MBN) were significantly affected by the highest dose of BDP and
246 soil type (Table 4). MBC at day 0 was 50% higher in the loamy soil than in the sandy soil
247 (Table 4). In both soils, after an initial decline in the first two months, MBC significantly
248 increased in the P10000 treatment (from days 112 to 350). In the loamy soil, this increase
249 in MBC was observed between days 56 and 168, and then the level decreased, although
250 values at the end of the incubation period were still 70% higher than those of the other
251 treatments. In the sandy soil, MBC increased with the P10000 treatment (+67%) until day
252 224 compared to the other treatments, and then tended to level off to the values of the
253 control and other treatments. A similar pattern was also observed for MBN (Figure 2). Even
254 for this parameter, from day 112 (Table 4), P10000 induced a significant increase in MBN
255 (+68 and +48% compared to the other treatments in the sandy and loamy soils, respectively)
256 with a pattern similar to that of MBC. The loamy soil showed significantly higher values
257 (+62%) than the sandy soil on days 56 and 168 (Table 4).
258 A pattern opposite to that of MBC and MBN was observed for TDN that showed continuous
259 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,

310 the addition of C substrate to soil, such as the that applied in our study, actually impacts
311 microbial activity and can cause either an acceleration of microbial biomass turnover
312 (apparent priming effect) or a change in the mineralisation of the SOM as a result of a real
313 priming effect (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000). Real priming is
314 usually observed with complex substrates poor in N, where microorganisms use native SOM
315 to recover energy and N for the synthesis of enzymes capable of metabolising the substrate.
316 Therefore, the N limitation induced by a surplus of complex C added to the soil stimulates N
317 mining from native SOM, which is one of the mechanisms responsible for the real priming
318 effect (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014). In our experiment, treatment
319 with the highest dose of BDP supplied a large amount of C (6 g kg_{ds}^{-1}) but not N, and in this
320 context, a real priming effect might have contributed to the increased SR. This increase,
321 however, was greater in the sandy soil, where the calculated $CO_{2,max}$ value was two times
322 higher than that of the loamy soil, supporting that the former has a higher C mineralisation
323 capacity over time than the latter. The lower release of CO_2 from the loamy soil after BDP
324 addition was also evident with the lower doses that caused a reduction in the CO_2 released
325 from soil compared to the control. Therefore, not only the amount of BDP and the related C
326 added to the soil is an important factor in terms of stimuli to SR, but soil characteristics also
327 play an important role in the regulation of this process (Sintim et al., 2019).
328
329 *4.2 Soil biochemical parameters*

330 In both soils at the end of the first month of incubation, most of the C added with the BDP
331 still remained in the system, potentially available to the soil microbial community. However,
332 only the highest BDP treatment supplied a dose of C able to stimulate a significant response
333 in terms of microbial biomass content, Dehy and Phos activities, and N immobilisation. The
334 increase in C availability with the P10000 treatment may have stimulated microbial growth

335 and activity and caused a decrease in TDN content (Sinsabaugh, 2010), which has become
336 the limiting element in this context (Li et al., 2014). The same result was observed by Li et
337 al. (2014), who attributed the N dynamic to the fact that biodegradable films (such as the
338 ones used in the present study) are generally C-rich but nutrient-poor. In particular, the BDP
339 tested in our experiment did not contain any N and, unlike the lower doses that did not affect
340 the C/N ratio of the soil, the highest dose caused a shift in soil C/N ratio from 10 to 14 in the
341 loamy soil and from 9 to 13 in the sandy soil (Table 2). Therefore, the demand for N by the
342 microbial biomass that we observed in our study might be driven either by this consistent
343 shift in C/N ratio of the soil towards higher values, or by the structural complexity of this kind
344 of material (Sinsabaugh, 2010). These two aspects affect the dynamics of microbial
345 biomass, N immobilisation, and enzymatic activities. According to the stoichiometric
346 decomposition theory, the C/N ratio of the substrate, compared to that of the microbial
347 biomass, drives the decomposition process and regulates the amount of C and N that is
348 used for microbial growth, which is released as CO_2 and mineral N (Barrett and Burke, 2000;
349 Hessen et al., 2004; Mooshammer et al., 2014b). The addition of the highest dose of BDP,
350 which is a C-rich substrate without N, increased the C content of the system but also caused
351 a stoichiometric imbalance between the substrate and microbial biomass, thereby leading
352 to the sequestration of soil available N by soil microorganisms. The demand for N during the
353 incubation period may have also stimulated the decomposition of SOM for N mining by the
354 microbial biomass (Moorhead and Sinsabaugh, 2006). Moreover, BDP are a complex
355 substrate that need to be deconstructed through the activity of extracellular enzymes to
356 make its C available to the microbial biomass (Caldwell, 2005). The production of
357 extracellular enzymes also requires N and can further contribute to N sequestration
358 (Mooshammer et al., 2014a; Schimel and Weintraub, 2003). In our study, we observed in
359 both soils a clear and significant effect of the BDP treatment on the N dynamics only after
360 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

387 impacted all enzymatic activities and MI (Table 4, Figure 3), with higher values in loamy soil.
388 This was not a surprising result, as it is known that enzymes are strongly adsorbed by clays,
389 which influence their activity and stability in the soil (Burns, 1982, 1978; Monreal and
390 Bergstrom, 2000; Saviozzi et al., 1997).
391 However, considering all these parameters (microbial biomass, MI, enzyme activities, and
392 available C and N) together in the PCA analysis (Figure 6) for both soils revealed a strong
393 relationship of P10000 treatment with microbial biomass (MBC and MBN) and activity (Dehy
394 activity), confirming that BDP addition strongly affected the microbial community
395 (Bandopadhyay et al., 2018) potentially by stimulating SOM degradation and N mining.
396
397 *4.3 Nitrification potential*
398 The nitrification potential, similar to most of the observed parameters, was much higher in
399 the loamy soil than in the sandy soil. The lower doses of BDP always showed similar results
400 to the control, and did not significantly affect the nitrification process. Our results with the
401 lower doses confirm the findings of Bettas Ardisson et al. (2014), who did not observe any
402 negative effect on the nitrification rate after tillage with BDP mulches.
403 A significant increase in the nitrification potential was observed only with the highest dose
404 of BDP in loamy soil at all sampling times. In sandy soil, this effect was observed only at the
405 first sampling time (day 112 of incubation) with P10000, which was 29% higher than that of
406 the other treatments; afterwards, the values became similar to the control.
407 As previously observed, the highest BDP dose stimulated a general increase in microbial
408 biomass and a greater N immobilisation as a result of the higher amount of C added. High
409 levels of MBN measured in soils receiving high C inputs were reported to be accompanied
410 by high rates of gross ammonification due to the high N demand (Burger and Jackson,
411 2003). As already hypothesised, in our study, the elevated and prolonged N demand during

412 the period of maximal microbial growth (days 56–224) may have stimulated the
413 mineralisation of SOM and the ammonification rate. In this phase, however, the N released
414 from SOM mineralisation may be simultaneously immobilised by the heterotrophic microbial
415 community that preferentially assimilates mineral N in the NH_4^+ form (Recous et al., 1990;
416 Rice and Tiedje, 1989; Shi et al., 2004; Shi and Norton, 2000) and is more competitive for
417 NH_4^+ compared to nitrifiers (Johnson, 1992; Recous et al., 1990; Schimel et al., 1989).
418 Consequently, despite the higher nitrification potential, the level of TDN remained
419 significantly lower than the other treatments. Moreover, we cannot exclude the possibility
420 that some NO_3^- may also be immobilised. Indeed, in the presence of a high amount of
421 complex C substrate, NO_3^- has been found to be immobilised to a certain extent (Burger and
422 Jackson, 2003; Cheng et al., 2017).
423

424 ## 5. CONCLUSIONS

425 The addition of C from BDP influenced the processes linked to the C and N cycles, with
426 positive effects on soil microbial biomass, even if the extent of the processes was
427 significantly influenced by the physicochemical characteristics of the soils considered.
428 Indeed, C and N dynamics and enzyme activities were strongly affected by soil texture,
429 independent of the BDP dose added.
430 The lower doses of BDP (P10, P100, and P1000) induced results that were comparable to
431 those of the control, indicating that their addition to the soil did not affect the soil
432 biochemistry. Only the highest dose of BDP (P10000) stimulated growth of the microbial
433 biomass, increased C mineralisation, and increased immobilisation of available N. Indeed,
434 addition of C with the highest BDP dose caused an imbalance in the C/N ratio, thereby
435 increasing the need for microorganisms to immobilise N, the limiting element. This, in turn,
436 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

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