

Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

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

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/). When citing, please refer to the published version.

(Article begins on next page)

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

2

- 3 Martina Mazzon*, Paola Gioacchini, Daniela Montecchio, Salvatore Rapisarda, Claudio
- 4 Ciavatta, Claudio Marzadori
- 5 Department of Agricultural and Food Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

6

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

8 ABSTRACT

In agriculture, the use of soil biodegradable mulch films could represent an eco-friendly 9 alternative to conventional plastic films. However, soil biodegradable mulch films 10 11 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-12 compatibility of these biodegradable plastics needs to be evaluated for their impact on 13 different soil functions. To understand the effect of biodegradable plastics on soil quality (i.e. 14 microbial biomass, nitrogen cycle, and activity of soil enzymes involved in the biochemical 15 processes of carbon and nitrogen), we added increasing quantities of biodegradable plastics 16 17 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 18 to carbon and nitrogen cycles. Significant effects were observed mainly with the highest 19 dose of biodegradable plastics added (1%), resulting in a higher growth of microbial 20 biomass, increased carbon mineralisation, and increased immobilisation of available 21 22 nitrogen. The results also underline the importance of evaluating the impact of biodegradable plastics in different soils because all the processes considered are also 23 influenced also by soil physicochemical characteristics. 24

25 Keywords

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

28

29 Highlights

- 30 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

35 **1. INTRODUCTION**

Plastics are durable and cost-efficient materials that have been applied in a wide range of 36 sectors, including agricultural production, particularly as plastic mulch (PlasticsEurope, 37 38 2018). In agriculture, plastic mulch contributes to increasing yields, extending the growing season, reducing weed pressure, improving fertiliser use efficiency, preserving soil moisture, 39 and increasing soil temperature (Lalitha et al., 2010; Lamont, 2005). One of the major 40 limitations to the use of plastic mulch is related to the operations and costs of removing and 41 disposing of mulch film from the field at the end of the crop cycle; indeed incorrect removal 42 and/or disposal of plastic mulch may cause environmental accumulation of fragmented 43 44 materials and subsequent pollution of soil, water, and air resources (Moore-Kucera et al., 2014; Steinmetz et al., 2016). 45

The use of biodegradable plastics (BDP) as mulch films could represent an eco-friendly 46 alternative to conventional plastic films. BDP mulch films offer the same agronomic 47 advantages as plastic mulch films, but do not need to be removed and disposed of at the 48 49 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 50 used and mineralized by soil microorganisms, leading to reduced environmental impact and 51 52 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 53 carbon (C), potentially influencing soil microbial biomass and activity. Consequently, these 54 processes influence the biogeochemical cycles of elements and their bioavailability. 55 Bandopadhyay et al. (2018) pointed out that the amount of C added to the soil for each 56 57 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; 58 Yamamoto-Tamura et al., 2015). Several studies concerning the effects of conventional 59

plastics on soil quality have been conducted taking into account physical, chemical, and 60 biological parameters; however, conflicting conclusions have been reached. For example, 61 Liu et al. (2017) studied the effect of polypropylene microplastics on the dynamics of soluble 62 forms of C and nitrogen (N) (DOC, dissolved organic C and TDN, total dissolved N) and on 63 soil enzyme activities. They found a stimulus of soil enzymatic activities that resulted in an 64 increased availability of soluble C for microorganisms and nutrients (N and phosphorous 65 (P)) for plants. In contrast, Awet et al. (2018) observed a reduction in soil dehydrogenase, 66 N-(leucine-aminopeptidase), P-(alkaline-phosphatase) and C-(β-glucosidase and cellulose 67 1,4-beta-cellobiosidase) activities after the incorporation of polystyrene nanoparticles into 68 69 the soil.

In the case of BDP, while biodegradation processes have been and are the subject of 70 numerous studies in terms of mechanism and kinetics (Chinaglia et al., 2018; Dharmalingam 71 72 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 73 studies have investigated the effects of these materials on soil functionality, with results that 74 are not always consistent (Bandopadhyay et al., 2018; Li et al., 2014; Qi et al., 2020; Sintim 75 et al., 2019). This is mainly because of the presence of different edaphic factors (i.e. 76 77 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 78 fungal abundance (Li et al., 2014; Muroi et al., 2016); however, Moreno and Moreno (2008) 79 80 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 81 82 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 83 enzyme activity (β -glu) in soil with BDP and no corresponding increase in microbial biomass. 84 Nonetheless, the effective eco-compatibility of these BDP needs to be evaluated by 85

targeting the impact on different soil functions, particularly those related to the supply of 86 87 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 88 microbial growth and activity, as well as the composition of the microbial community. This, 89 in turn, would influence the enzymatic activities directly involved in BDP degradation and/or 90 in the release of nutrients needed for microbial growth and for the synthesis of the enzymes 91 responsible for the degradation. Together, these mechanisms can affect the cycle of 92 nutrients and their availability to plants; therefore, the soil functions are directly related to 93 soil fertility (Bastida et al., 2008; Giacometti et al., 2013; Gil-Sotres et al., 2005; Mazzon et 94 95 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.

102

103 **2.**

2. MATERIALS AND METHODS

104 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), 122 and 10,000 (P10000) mg of biodegradable plastic per kg of dry soil (Table 2). The P100 123 treatment (100 mg/kg soil) corresponds to the mean annual quantity of BDP material 124 incorporated into the soil (EN 17033 suggests 0.0063% = 63 mg/kg calculated based on 125 mean characteristics of BDP), whereas the P10000 treatment corresponds to a loading rate 126 of 1%, the quantity recommended in EN 17033. The BDP were added as small fragments 127 (< 2 mm) and carefully mixed with the soil. The containers were covered with screw caps 128 with a few holes to ensure gas exchange during incubation. The moisture content of each 129 container was checked weekly and restored when necessary. 130

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.

134

135 2.2 Soil respiration

Soil respiration was simultaneously measured for 35 days with a distinct incubation 136 developed at 23 °C. For this analysis, moist soil samples, equivalent to 10 g of dry soil, were 137 weighed in aluminium vessels, and the amount of BDP corresponding to the different 138 treatments (Table 2) were added to the soil. The samples were then placed within airtight 139 glass jars together with a glass vial containing 20 mL of 0.25 M NaOH. Twice a week, the 140 vials were changed to new vials. Carbon dioxide (CO₂) released from the soil and trapped 141 by NaOH was quantified using an elemental analyser for liquid samples (TOC-VCPH/CPN, 142 Shimadzu Corp., Kyoto, Japan) and expressed as µg C-CO₂ g_{ds}⁻¹ (Cheng, 2009). 143

144

145 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 kgds⁻¹.

Three enzymatic activities were measured during the experiment: dehydrogenase (Dehy) 151 and β -glucosidase (β -glu) involved in the C cycle, and alkaline phosphatase (Phos) linked 152 to P availability. The activity of these enzymes can be used as good indicators of soil quality 153 and functionality (Gil-Sotres et al., 2005; Rao and Gianfreda, 2014). Dehy and β -glu are 154 linked to the C cycle: the former (an intracellular enzyme) plays a marked role in the 155 biological oxidation of SOM by transferring hydrogen from organic substrates to inorganic 156 acceptors (Kumar et al., 2013), and the latter (an extracellular enzyme) hydrolases maltose, 157 cellobiose and related products, which are important sources of energy for soil 158 microorganisms (Ferraz De Almeida et al., 2015; Zhang et al., 2011). Both Dehy and β -glu 159 are considered to be good soil quality indicators related to soil microbial activity (Dick and 160

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).

165 Dehy activity was determined according to the method described by von Mersi and Schinner 166 (1991a). Moist soil (1g) was incubated with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-167 phenyltetrazolium chloride (INT) at 37 °C for 2 h. The release of 5-(4-iodophenyl)-1-(4-168 nitrophenyl)-3-phenylformazan (INTF) was measured at 464 nm and dehydrogenase activity 169 was expressed as μ g INTF gds⁻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).

183

184 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-NO₂- g_{ds}-1 h-1. The nitrification potential was measured at 112, 224, 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).

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

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

215

216 **3. RESULTS**

217 3.1 Soil respiration

The CO₂ released during the first month of incubation, the calculated asymptotic maximum 218 quantity of CO₂ produced, and the k parameter are listed in Table 3. The addition of BDP at 219 lower doses (P10, P100, and P1000) induced different soil respiration (SR) responses in the 220 two soils. In the loamy soil, SR was reduced compared to the control, whereas in the sandy 221 soil, there was an increase in SR with dose, with 11%, 27%, and 40% SR for P10, P100, 222 223 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 224 other treatments was P10000 (Table 3). However, this increase was different between the 225 two soils: in the loamy soil, only 49% more CO₂ was released compared to the control, 226 whereas in the sandy soil, the extra CO₂ release was 435%. 227

In general, the loamy soil showed values of SR much closer to those of the calculated asymptotic maximum quantity of CO₂ 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 CO₂ release.

235

236 3.2 Soil biochemical parameters

The changes in soil biochemical parameters over time during the incubation period are 237 238 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 239 the sandy soil, the observed fluctuations may represent cycles of immobilisation and release 240 of DOC in the system, with values always higher than the initial values (Figure 2). Moreover, 241 the sandy soil showed significantly higher values (+45% on average) of DOC than loamy 242 soil for all measurement times. However, the behaviour of DOC was not influenced by BDP 243 244 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 245 soil type (Table 4). MBC at day 0 was 50% higher in the loamy soil than in the sandy soil 246 (Table 4). In both soils, after an initial decline in the first two months, MBC significantly 247 increased in the P10000 treatment (from days 112 to 350). In the loamy soil, this increase 248 249 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 treatments. In the sandy soil, MBC increased with the P10000 treatment (+67%) until day 251 252 224 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 253 for this parameter, from day 112 (Table 4), P10000 induced a significant increase in MBN 254 (+68 and +48% compared to the other treatments in the sandy and loamy soils, respectively) 255 with a pattern similar to that of MBC. The loamy soil showed significantly higher values 256 257 (+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 263 that were more accentuated in the loamy soil. Specifically, the main differences in Phos 264 activity occurred from day 112 with loamy soil (Table 4), which showed higher values than 265 the sandy soil (+59% on average) until the end of the experiment (day 350). In the loamy 266 soil, P10000 treatment induced higher Phos activity than the other treatments at days 168 267 and 224 (+20%), whereas in the sandy soil, higher Phos activity occurred at days 112, 168, 268 269 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 270 type from days 0 to 350 (Table 4), with higher values in the loamy soil (β -glu +73%, Dehy 271 272 +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 273 in both soils only on day 112. Dehy showed significant differences mainly for the soil type -274 BDP interaction (Table 4); BDP effects were comparable between the two soils (Figure 3) 275 only on days 168 and 224, with P10000 that was higher (+28%) compared to the other 276 277 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 278 168. 279

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.

293 3.3 Nitrification potential

294 Changes in nitrification potential over time (Table S2) during the incubation period are shown 295 in Figure 7. In general, loamy soil showed a higher nitrification potential than sandy soil 296 (Figure 7, Table 6). The P10000 BDP dose increased the nitrification potential by 26%, 297 compared to the other treatments, in the loamy soil during the whole incubation period, 298 whereas in the sandy soil, an increase of 29% was detected only at the first sampling time, 299 corresponding to day 112 of incubation (Figure 7). Afterwards, in the sandy soil, the 200 nitrification potential significantly decreased and levelled off in all treatments.

301

302 4. DISCUSSION

303 4.1 Soil respiration

During the first month of incubation, only the highest dose of BDP (1%) caused an increase in CO₂ 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 CO₂ 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 CO₂, 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 310 microbial activity and can cause either an acceleration of microbial biomass turnover 311 (apparent priming effect) or a change in the mineralisation of the SOM as a result of a real 312 priming effect (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000). Real priming is 313 usually observed with complex substrates poor in N, where microorganisms use native SOM 314 to recover energy and N for the synthesis of enzymes capable of metabolising the substrate. 315 Therefore, the N limitation induced by a surplus of complex C added to the soil stimulates N 316 mining from native SOM, which is one of the mechanisms responsible for the real priming 317 effect (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014). In our experiment, treatment 318 319 with the highest dose of BDP supplied a large amount of C (6 g kgds⁻¹) but not N, and in this context, a real priming effect might have contributed to the increased SR. This increase, 320 however, was greater in the sandy soil, where the calculated CO_{2,max} value was two times 321 higher than that of the loamy soil, supporting that the former has a higher C mineralisation 322 capacity over time than the latter. The lower release of CO2 from the loamy soil after BDP 323 addition was also evident with the lower doses that caused a reduction in the CO2 released 324 from soil compared to the control. Therefore, not only the amount of BDP and the related C 325 added to the soil is an important factor in terms of stimuli to SR, but soil characteristics also 326 327 play an important role in the regulation of this process (Sintim et al., 2019).

328

329 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 335 the limiting element in this context (Li et al., 2014). The same result was observed by Li et 336 al. (2014), who attributed the N dynamic to the fact that biodegradable films (such as the 337 ones used in the present study) are generally C-rich but nutrient-poor. In particular, the BDP 338 tested in our experiment did not contain any N and, unlike the lower doses that did not affect 339 the C/N ratio of the soil, the highest dose caused a shift in soil C/N ratio from 10 to 14 in the 340 loamy soil and from 9 to 13 in the sandy soil (Table 2). Therefore, the demand for N by the 341 microbial biomass that we observed in our study might be driven either by this consistent 342 shift in C/N ratio of the soil towards higher values, or by the structural complexity of this kind 343 344 of material (Sinsabaugh, 2010). These two aspects affect the dynamics of microbial biomass, N immobilisation, and enzymatic activities. According to the stoichiometric 345 decomposition theory, the C/N ratio of the substrate, compared to that of the microbial 346 347 biomass, drives the decomposition process and regulates the amount of C and N that is used for microbial growth, which is released as CO₂ and mineral N (Barrett and Burke, 2000; 348 Hessen et al., 2004; Mooshammer et al., 2014b). The addition of the highest dose of BDP, 349 which is a C-rich substrate without N, increased the C content of the system but also caused 350 a stoichiometric imbalance between the substrate and microbial biomass, thereby leading 351 352 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 353 microbial biomass (Moorhead and Sinsabaugh, 2006). Moreover, BDP are a complex 354 substrate that need to be deconstructed through the activity of extracellular enzymes to 355 make its C available to the microbial biomass (Caldwell, 2005). The production of 356 extracellular enzymes also requires N and can further contribute to N sequestration 357 (Mooshammer et al., 2014a; Schimel and Weintraub, 2003). In our study, we observed in 358 both soils a clear and significant effect of the BDP treatment on the N dynamics only after 359 two months of incubation, when N immobilisation clearly started in both soils in the presence 360

of the highest BDP dose (Table 4, Figure 2). This caused significant microbial growth in both 361 soils from day 112, which remained high until day 224. At approximately the same time 362 range (days 112–224), the highest levels of dehydrogenase and phosphatase activities were 363 observed. The increased Dehy activity could be related to the SOM degradation (N mining) 364 induced by the demand for N (Kumar et al., 2013; Piotrowska-Długosz, 2014; Srinivasulu 365 and Rangaswamy, 2014). Moreover, Phos activity could be related to the demand for P by 366 367 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, 368 a net N release was observed with P10000 treatment in both soils with TDN, which increased 369 370 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%, 371 and the same trend was observed for the abovementioned enzymatic activities. Together, 372 373 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 374 death of some of the soil microorganisms and their subsequent turnover (Bello et al., 2014). 375 The trend over time observed for microbial biomass, TDN, and Dehy and Phos activities 376 was not observed for β -glu activity and MI. Contrary to our results, Li et al. (2014) observed 377 an increase in β-glu activity without a corresponding increase in microbial biomass, 378 suggesting that there was a more efficient metabolic process for the microbial community 379 and that β -glu activity is a responsive parameter for testing mulch effects on soil. In our 380 study, we did not observe any marked increase in β -glu activity with P10000 treatment. This 381 could be attributed to the complexity of BDP as substrates, which may reach a degree of 382 degradation that is not sufficient to bring biodegradation products suitable for β -glu utilisation 383 to the soil. Indeed, it is known that β -glu is mainly involved in the last stage of degradation 384 of C-substrates as glucosidases hydrolyse the degradation products of amylase and 385 cellulose (Deng and Popova, 2011; Piotrowska-Długosz, 2014). The soil type significantly 386

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.

396

397 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 412 mineralisation of SOM and the ammonification rate. In this phase, however, the N released 413 from SOM mineralisation may be simultaneously immobilised by the heterotrophic microbial 414 community that preferentially assimilates mineral N in the NH₄⁺ form (Recous et al., 1990; 415 Rice and Tiedje, 1989; Shi et al., 2004; Shi and Norton, 2000) and is more competitive for 416 NH4⁺ compared to nitrifiers (Johnson, 1992; Recous et al., 1990; Schimel et al., 1989). 417 Consequently, despite the higher nitrification potential, the level of TDN remained 418 significantly lower than the other treatments. Moreover, we cannot exclude the possibility 419 that some NO₃⁻ may also be immobilised. Indeed, in the presence of a high amount of 420 421 complex C substrate, NO₃⁻ has been found to be immobilised to a certain extent (Burger and Jackson, 2003; Cheng et al., 2017). 422

423

424 **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).

437	Our study has clearly shown that, independent of soil physical-chemical characteristics, BDP
438	addition at higher dose (1%) induces an imbalance in C/N stoichiometry, which opens the
439	road for future investigation that should include plant and N supply in order to evaluate BDP
440	effects on both soil and plants.
441	

442 CRediT authorship contribution statement

- 443 Mazzon: methodology, formal analysis, writing, review and editing
- 444 Gioacchini: conceptualization, investigation, writing, review and editing
- 445 Montecchio: conceptualization, investigation, review and editing
- 446 Rapisarda: investigation, methodology, writing
- 447 Ciavatta: conceptualization, supervision, funding acquisition
- 448 Marzadori: conceptualization, supervision, funding acquisition
- 449

450 Funding sources

- 451 This research was supported by Novamont S.p.A..
- 452

453 **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.
- 456 Awet, T.T., Kohl, Y., Meier, F., Straskraba, S., Grün, A.L., Ruf, T., Jost, C., Drexel, R., Tunc,
- 457 E., Emmerling, C., 2018. Effects of polystyrene nanoparticles on the microbiota and 458 functional diversity of enzymes in soil. Environ. Sci. Eur. 30. 459 https://doi.org/10.1186/s12302-018-0140-6
- Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., 2018. Biodegradable

- 461 plastic mulch films: Impacts on soil microbial communities and ecosystem functions.
 462 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 472 rates in two swedish arable soils. Biol. Fertil. Soils 1. 131–140. 473 https://doi.org/10.1007/BF00301780 474
- 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

484	Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in
485	relation to ammonification and nitrification rates in organic and conventional cropping
486	systems. Soil Biol. Biochem. 35, 29–36. https://doi.org/10.1016/S0038-0717(02)00233-
487	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

490 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
- 496 Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya,

497 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
- 503 Cheng, W., 2009. Rhizosphere priming effect: Its functional relationships with microbial 504 turnover, evapotranspiration, and C-N budgets. Soil Biol. Biochem. 41, 1795–1801.
- 505 https://doi.org/10.1016/j.soilbio.2008.04.018
- 506 Chinaglia, S., Tosin, M., Degli-Innocenti, F., 2018. Biodegradation rate of biodegradable

507	plastics	at	molecular	level.	Polym.	Degrad.	Stab.	147,	237–244.	
508	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
- 513 Deng, S., Popova, I., 2011. Carbohydrate hydrolases., in: Dick, R.P. (Ed.), Methods of Soil
 514 Enzymology. SSSA Book series 9.

515 Dharmalingam, S., Hayes, D.G., Wadsworth, L.C., Dunlap, R.N., DeBruyn, J.M., Lee, J.,

516 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

- 519 Dick, W.A., Tabatabai, M.A., 1992. Significance and potential uses of soil enzymes, in: 520 Metting, F.B. (Ed.), Soil Microbial Ecology - Applications in Agricultural and 521 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

524 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.

530 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.

541 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
- 552 Kumar, S., Chaudhuri, S., Maiti, S.K., 2013. Soil dehydrogenase enzyme activity in natural

- Sci. Res. 13. 898-906. 553 and mine soil -Α review. Middle East J. https://doi.org/10.5829/idosi.mejsr.2013.13.7.2801 554
- 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/S00380717(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.,
- 571 2008. Polymer biodegradation: Mechanisms and estimation techniques A review.
 572 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

576 Masciandaro, G., Ceccanti, B., Ronchi, V., Bauer, C., 2000. Kinetic parameters of 577 dehydrogenase in the assessment of the response of soil to vermicompost and 578 inorganic fertilisers. Biol. Fertil. Soils 32, 479–483. 579 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., ZechmeisterBoltenstern, 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

598 Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014b. 599 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).
- 604 116, 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
- 618 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-

623 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–
- 645 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
- 650 Sintim, H.Y., Bandopadhyay, S., English, M.E., Bary, A.I., DeBruyn, J.M., Schaeffer, S.M.,

651

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
- 654 Srinivasulu, M., Rangaswamy, V., 2014. Enzymes and pesticides, in: Enzymes in 655 Agricultural Sciences; Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.
- 656 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/10.1016/00380717(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.
- 677 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