

1 Running title *Linking Tea Bag Index to soil properties and land use*

2 **Core ideas**

- 3 • Litter decomposition, quantified by the Tea Bag Index (TBI), was determined across land
- 4 uses
- 5 • Grasslands had the highest decomposition rate and stabilization factor of plant litter
- 6 • Soil gas transport was more important to stabilization factor, not decomposition rate
- 7 • Variability in the TBI parameters was affected by soil pH, P_{oxalate} and bulk density
- 8 • The inclusion of land use improved the predictions of both TBI parameters

9

10 **Linking Litter Decomposition to Soil Physicochemical Properties, Gas Transport and Land**

11 **Use**

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20 Abbreviations: Al_{ox} , oxalate-extractable aluminum; D_0 , oxygen diffusion in free air; D_p , oxygen
21 diffusion in soil; D_p/D_0 , relative gas diffusivity; ε , air-filled porosity; EC, electrical conductivity;
22 Fe_{ox} , oxalate-extractable iron; k , decomposition rate; k_a , air permeability; ϕ , total porosity; P_{ox} ,
23 oxalate-extractable phosphorus; ρ_b , bulk density; ρ_s , particle density; RMSE, root mean square

24 error; S , stabilization factor; SOC, soil organic carbon; SOM, soil organic matter; TBI, Tea Bag
25 Index.

26

27

ABSTRACT

28 Litter decomposition is a critical process in carbon cycling, which can be impacted by land use.
29 The relationship between litter decomposition and soil properties under different land uses
30 remains unclear. Litter decomposition can be quantified by the Tea Bag Index (TBI), which
31 includes a decomposition rate k , and a stabilization factor S . Our objective was to investigate
32 linkages between TBI and soil physicochemical and gas transport properties and land use. We
33 buried three pairs of tea bags in 20 sites (covering cropland, grassland, heathland, and forest land
34 uses) in a transect from the western to the eastern coast of the Jutland peninsula, Denmark. The
35 tea bags were retrieved after 90 days and TBI was determined. Disturbed and undisturbed (100
36 cm^3 soil cores) samples were collected from each site. Thereafter, clay content, organic carbon,
37 bulk density, pH, electrical conductivity, oxalate-extractable phosphorus (P_{ox}), aluminum and
38 iron content, soil water content, gas diffusivity (D_p/D_0) and air permeability (k_a) at -10 kPa were
39 measured. Results showed that grasslands had the highest k and S among four land uses, and
40 agricultural soils (croplands and grasslands) exhibited higher TBI values than semi-natural soils
41 (forest and heathland). The prediction of S was better than that of k based on multiple linear
42 regression analysis involving soil physicochemical properties. Clay content and organic carbon
43 were not strong predictors. Including D_p/D_0 and k_a improved the prediction of S , and finally, the
44 inclusion of land use enhanced the prediction of both k and S . The different trends between two
45 distinct land-use groups can be attributed to pH, P_{ox} and bulk density.
46 Keywords: Tea Bag Index, Soil gas diffusivity, Soil air permeability

INTRODUCTION

47

48 The decomposition of soil organic matter (SOM), the fundamental process in carbon cycling, is
49 controlled by three main factors: substrate quality, microbial community composition and soil
50 environment (Swift et al., 1979). Plant litter is one of the main SOM sources and the primary
51 rate-determining factors for litter decomposition depend on environmental conditions. Under
52 similar climatic conditions, litter quality and soil nutrients such as available P are believed to
53 have an overriding influence on litter decomposition (Prescott, 2010). Litter with high quality
54 (low C:N) tend to decompose faster than low-quality litter (high C:N). Nevertheless, the
55 persistence and degradability of SOM are argued to be primarily controlled by the interaction
56 between SOM and its immediate environment rather than by the molecular property of the
57 substrate per se (Schmidt et al., 2011).

58 Soil physical and chemical properties such as texture, bulk density, pH, electrical
59 conductivity (EC) and nutrient availability control the soil environment and have an important
60 role in litter decomposition. For example, clayey soils contribute more to C stabilization than
61 sandy soils (Angst et al., 2021) and this can be attributed to the high fraction of small pores in
62 clayey soils. Due to the heterogeneity of the soil matrix, the access of soil microorganisms to
63 substrate and oxygen affects the activity of microbes and in turn the decomposition of SOM
64 (Tecon and Or, 2017). The ability of soil microorganisms to mineralize SOM differs at different
65 soil pore size scales. Anaerobic microsites constrain oxygen supply and may preserve certain
66 organic compounds in the long term and benefit organic carbon (OC) stabilization (Keiluweit et
67 al., 2018), while larger pores ($>180\mu\text{m}$) are often drained faster and thus may be unfavorable for
68 microbial activities (Kravchenko et al., 2019). In terms of soil pH, acidic soils may limit soil
69 microbial growth and lead to less litter decomposition and accumulation of SOC (Malik et al.,

70 2018). High soil EC can also cause stress on microbial communities and suppress microbial
71 activity (Yuan et al., 2007). Soil nutrients such as N and P are fundamental for microbial growth
72 and P is usually a limiting nutrient in forests as its source is mainly rock weathering and
73 independent of the climate (Augusto et al., 2017). One source of plant-available P is adsorbed P
74 on the soil surface, which is strongly related to oxalate extractable Al and Fe oxides. Moreover,
75 Al- and Fe- oxyhydroxides are also good predictors of SOM content in acidic and moist soils
76 (Rasmussen et al., 2018).

77 Land use has a strong impact on soil physical and chemical properties, and in the context
78 of global climate change, the impact of land-use change on C storage is a concern. For example,
79 the conversion of forests to cropland leads to about a 40% decline in carbon stocks (Guo and
80 Gifford, 2002). Given the fact that C stock is crucial for food security and various ecosystem
81 services, understanding the relationships between land use, soil properties and SOM
82 decomposition is critical and far from fully understood. The management practices for cropland
83 and grassland, including fertilization and/or tillage, enrich the nutrient pool and alter soil
84 structure (Compton and Boone, 2000; Hebb et al., 2017), making them distinct from natural
85 lands. Tilled soils have a more homogenous topsoil layer with lower mechanical impedance
86 which benefits root growth, while natural lands tend to have more macropores from the high
87 biological activity and the legacy of decayed roots (Or et al., 2021). As a result of management,
88 croplands tend to exhibit a higher tendency to decompose SOM than other land use (Becker and
89 Kuzyakov, 2018). Conversely, a lower litter decomposition rate was reported in croplands
90 compared to a less compacted grass margin that was sown adjacent to the cropland (Carlesso et
91 al., 2019), which is likely due to the compaction-induced change of gas transport paths in
92 cropland soil. Although converting croplands into grasslands has been seen as a way to promote

93 C storage, the amount of C retained in grassland is also strongly dependent on management
94 (Yeasmin et al., 2020).

95 Litter bags have long been used in the study of SOM decomposition (e.g. Bocoock &
96 Gilbert, 1957). To reduce the tediousness associated with the use of litter bags, Keuskamp et
97 al.(2013) described a simple approach to assess litter decomposition using two types of
98 commercially available tea as standardized plant litter. The weight loss data from the two tea
99 types are utilized to calculate the Tea Bag Index (TBI), which is characterized by two
100 parameters: decomposition rate k and stabilization factor S . The k is the exponential
101 decomposition rate of the early decomposition stage and the S refers to the part of labile
102 compounds stabilized and transformed into relatively recalcitrant compounds in given
103 environmental conditions. Unlike local plant litter which could be subjected to the environmental
104 filters, the tea leaves as standardized litter enable the comparison between land uses and soil
105 types (e.g. Becker and Kuzyakov, 2018; Toleikiene et al., 2020). The TBI protocol has shown a
106 capacity to be widely applied, from a regional to a global scale (e.g. Djukic et al., 2018).

107 In this study, we aimed to explore the linkages between the TBI and the aforementioned
108 soil physicochemical properties and land use on a local scale. We considered a transect of 20
109 points covering four common land uses (cropland, grassland, heathland and forest) from the
110 western to the eastern coast of the Jutland peninsula, Denmark. The climate throughout the
111 transect had little variation and thus allowed us to study the effect of land use and soil physical
112 properties on the litter decomposition without the confounding effects of climate. We
113 hypothesized that k and S would vary distinctly with land use and both could be accurately
114 predicted from soil physicochemical properties combined with land use. The objectives of this
115 study were 1) to determine the soil physicochemical properties of the four land uses, 2) to

116 quantify the litter decomposition and stabilization of different land uses, and 3) to analyze the
117 effect of soil properties, soil gas transport and land use on the TBI k and S parameters.

118

119

MATERIALS AND METHODS

Description of study sites

121 The study included 20 study sites that are distributed from the eastern coast to the western coast
122 of central Jutland, Denmark between 8°8'E to 10°43'E and 56°15'N to 56°29'N (Fig. 1). The
123 climate in this area is Temperate Continental. The litter decomposition study was conducted
124 between March and June 2017. The average temperature and precipitation during the study
125 period in the study area were $10.9 \pm 0.1^\circ\text{C}$ and 61.7 ± 2.7 mm, respectively (Danish
126 Meteorological Institute). There was minimal variation of temperature throughout the sites while
127 the rainfall varied with the location. The west coast had the least average daily precipitation with
128 an average value of 53 mm during the studied period, while the inland area, i.e., Randers had the
129 highest value 68.6 mm (Fig. S1).

130 The study included four types of land use: cropland, grassland, heathland and forest. In
131 croplands, winter cereals (w. wheat, *Triticum aestivum* L.; w. hybrid rye; w. barley, *Hordeum*
132 *vulgare* L.) were grown during the study period. The fields were ploughed and seeds were sown
133 in September the previous year and the fertilization was done in split rates between March and
134 May. The source of fertilizer was combined manure with mineral fertilizer. Averages of between
135 104 to 201 kg N/ha at farm level were applied in the fields and the specific dose for each field
136 followed the suggestion given by the Danish authority for the given fields based on the soil
137 condition, crop type, manure availability and the desired yield. The phosphorus fertilizer was
138 NPK fertilizer and the amount varied with plant type. Site #7 received the least amount of NPK

139 fertilizer among all sites. The rotation elements in the cropland were common cereals including
140 the aforementioned winter cereals, spring barley and spring oats. Site #8 had winter rape grown
141 in one of the past five years and spinach in one year. Site #4 had winter wheat grown for five
142 consecutive years. The grasslands were kept for grazing and fertilized with manure and/or
143 mineral fertilizer, except for the one with clover which received no fertilizer. The heathlands
144 were dominated by heather (*Calluna vulgaris*). In forests, Scots pine (*Pinus sylvestris*), birch
145 (*Betula pubescens/Betula pendula*), willow (*Salicaceae*) and European beech (*Fagus sylvatica*)
146 were the dominant species. Based on the extent of human activities involved in these lands, we
147 grouped the four land uses into two groups, agricultural (cropland and grassland) and semi-
148 natural (heathland and forest) groups. Detailed information on each site is listed in Table 1.

149

150 *Tea Bag Index*

151 The tea bag incubation protocol from Keuskamp et al. (2013) was followed in this
152 decomposition study. Briefly, three pairs of Lipton green tea bags and rooibos tea bags were
153 buried at depths of 8 cm and 15 cm apart and retrieved after three months. The tea bags buried in
154 the western area were buried and retrieved one week earlier than the ones in the eastern area. But
155 all tea bags were incubated in the fields for the same amount of time. Plant roots and dirt were
156 carefully removed by hand and the remaining tea was extracted from the tea bags and dried at
157 60°C for 48 h. The Tea Bag Index (k and S) was calculated from the remaining mass and the
158 mass loss of tea. The k here is the decomposition rate of labile fraction and it was assumed that
159 the decomposition of the recalcitrant fraction was very slow and thus negligible in the short field
160 incubation (Keuskamp et al., 2013). The equations are given by Keuskamp et al. (2013):

$$161 \quad W_r(t) = a_r e^{-kt} + (1 - a_r) \quad (1)$$

162 where $W_r(t)$ is the mass of remaining rooibos tea after the incubation time t days, a_r is the labile
163 fraction of rooibos tea and $(1-a_r)$ is the recalcitrant fraction. The a_r is calculated from the
164 hydrolysable fraction of rooibos tea H_r (the value is given as 0.552 g g^{-1}) and S :

$$165 \quad a_r = H_r(1 - S) \quad (2)$$

166 The k was therefore calculated from a_r and $W_r(t)$ by using equation (1).

167 After three-month incubation, the labile compounds in green tea were assumed to be
168 decomposed (Keuskamp et al., 2013). It was assumed that the S was the same for both green tea
169 and rooibos tea. Thus, the calculation of S was based on the mass loss of green tea, namely, the
170 decomposable fraction of green tea a_g , and the hydrolysable fraction of green tea H_g whose value
171 is given as 0.842 g g^{-1} :

$$172 \quad S = 1 - \frac{a_g}{H_g} \quad (3)$$

173

174 *Soil sampling and analysis*

175 For the determination of the soil physicochemical properties, three undisturbed soil cores (100
176 cm^3 , 6.1 cm in diameter, and 3.4 cm in height) and bulk soil were collected from 5 – 15 cm soil
177 layer near the tea bag burial spots at each site during tea bag burial time. The extracted soil cores
178 were sealed and stored at 2°C before laboratory measurements. Bulk soil was air-dried, ground,
179 and sieved through 2 mm mesh for the measurements of soil texture and other properties. Soil
180 texture was determined by a combined hydrometer and wet sieving method after the removal of
181 soil organic matter with hydrogen peroxide (Gee and Or, 2002). Soil organic carbon was
182 determined on ball-milled aliquots by oxidizing C at 950°C with a Thermo Flash 2000 NC Soil
183 Analyzer (Thermo Fisher Scientific, USA). Oxalate-extractable phosphorus, aluminum and iron
184 (P_{ox} , Al_{ox} and Fe_{ox}) were measured using protocols described by Schoumans (2000). Soil pH was

185 measured using a pH meter (PHM220, Radiometer Analytical SAS, Lyon) in a soil suspension of
186 8 cm³ soil in 30 mL deionized water. Soil EC was measured using an EC meter (CDM210,
187 Radiometer Analytical SAS, Lyon) in a soil suspension of 4 g soil in 36 mL deionized water.

188 To determine the soil water retention and gas transport properties, the soil cores were
189 saturated in sandboxes with capillary water from beneath for three days and then drained to the
190 matric potential at field capacity (−10 kPa). Soil gas diffusivity (D_p) was determined by the one
191 chamber non-steady-state method (Taylor, 1950), with the setup and the detailed procedure
192 described by Schjønning et al. (2013). Briefly, the soil core was placed at the bottom of a
193 chamber which allowed free air to diffuse in the chamber through the soil core. The chamber was
194 flushed with N₂ before the measurements. The readings of O₂ concentration were recorded for 2
195 h in a 20°C lab. A value of 0.205 cm² s^{−1} was used for gas diffusivity of O₂ in free air (D_0) to
196 calculate the relative gas diffusivity (D_p/D_0).

197 Soil air permeability (k_a , μm²) was measured by the Forchheimer approach, with the
198 setup and the procedure described by Schjønning & Koppelgaard (2017). Briefly, compressed air
199 at four different pressure values (5, 2, 1, and 0.5 hPa) was applied to the soil core in a
200 measurement chamber, and the resulting air flows were measured. The Darcian k_a was calculated
201 from the Forchheimer polynomial regression using the four pressure and flow values.

202 After the gas transport measurements, the soil cores were oven-dried at 105°C for 24 h to
203 determine the oven-dried mass. The bulk density (ρ_b) was estimated as the ratio of oven-dried
204 mass to the total volume of the soil core. The soil particle density (ρ_s) was estimated based on the
205 clay and SOM content using the model given by Schjønning et al. (2017). The total porosity (ϕ ,
206 m³ m^{−3}) was calculated from ρ_b and ρ_s . The air-filled porosity (ε , m³ m^{−3}) was calculated from ϕ
207 and volumetric water content.

208 To compare the soils under different land use, the macroporosity-dependent model
209 developed by Moldrup et al. (2000) at matric potential -10kPa was used:

$$210 \frac{D_{p,10}}{D_0} = 2\varepsilon_{10}^3 + 0.04\varepsilon_{10} \quad (4)$$

211 where $D_{p,10}/D_0$ and ε_{10} are the relative gas diffusion and air-filled porosity at a matric potential
212 of -10 kPa , respectively.

213

214 *Statistical analyses*

215 The statistical analyses were carried out in R software version 4.0.5 (R Core Team, 2021).

216 Before the statistical analyses, normality and equal variance of the data were evaluated using the

217 Shapiro Wilk and Bartlett's tests, respectively. The Pearson correlation coefficient was used to

218 analyze the correlation between TBI parameters and basic soil properties. The criterion of

219 significance was $p < 0.05$. Multiple linear regression (MLR) was conducted to analyze the effect

220 of physicochemical soil properties, gas transport parameters and land use on k and S . The

221 variables for the MLR were selected by stepwise regression from both directions using the

222 "MASS" package. The first subset of variables were eight soil properties, including clay, OC, pH,

223 EC, bulk density, Al_{ox} , Fe_{ox} , P_{ox} . The second subset included the first subset and two gas

224 transport parameters, i.e. k_a and D_p/D_0 , and the third subset added land use to the second subset.

225 The selection of variables was based on AIC scores and the adjusted R^2 of MLR. The final model

226 from each subset contained the least number of variables with the best prediction. To evaluate

227 the fitting models with the measured data, the RMSE was used for $n=20$ data points:

$$228 \text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (\text{predicted}_i - \text{measured}_i)^2} \quad (5)$$

229

RESULTS AND DISCUSSION

230
231 Among the 20 sites, 19 sites had sandy soil with clay content ranging from 1 to 14 g 100g⁻¹
232 (Table 1). The average OC content for these 19 samples was 1.80 ± 0.14 g 100g⁻¹. One cropland
233 site (#8) was peat soil and it had the highest clay content and high OC content, with a resultant
234 very low bulk density of 0.64 g cm⁻³. Site #15 was a heathland on the west coast, and it had the
235 lowest OC and the highest sand content among all sites. Despite the small differences in the soil
236 texture between sites (except for #8), heathlands had the highest average bulk density (1.43 ±
237 0.04 g cm⁻³), followed by croplands (1.39 ± 0.03 g cm⁻³), grasslands (1.32 ± 0.03 g cm⁻³), and
238 forests (1.20 ± 0.02 g cm⁻³). The peat site #8 was excluded from the calculation of the bulk
239 density averages due to its very low bulk density. A similar trend was found in two German sites,
240 where the topsoil of croplands had higher bulk density than grasslands and forest, and forest soils
241 had the lowest bulk density (Bormann and Klaassen, 2008). The high bulk density of heathland
242 was because of the high sand content. Based on the similarity in soil management among the
243 four land uses, cropland and grassland were grouped as agricultural soils, while heathland and
244 forest were grouped as semi-natural soils. Agricultural soils were less acidic (pH~6.2) than the
245 semi-natural soils (pH~4.6) (Table 1). Several studies also found forest soils had lower pH than
246 agricultural soils (e.g. Falkengren-Grerup et al., 2006; Wiesmeier et al., 2012), which may be
247 explained by the leaching and the lack of calcium input in forest soils (Yesilonis et al., 2016).
248 Agricultural practices such as liming practice are often used in the acidic agricultural soils for
249 securing crop yield, and the manure addition has a liming effect on acidic soils (Ozlu and Kumar,
250 2018). Agricultural soils had higher EC than the semi-natural land uses, except for two outliers
251 in grassland and forest, with average values of 0.70 ± 0.14 and 0.35 ± 0.07 mS cm⁻¹,
252 respectively. Ozlu & Kumar (2018) reported that fields with manure input had higher EC than

253 mineral fertilizer and control treatments, which can be attributed to the dissolved salts in manure
254 (Azeez and van Averbek, 2012). Likewise, due to fertilization, higher P_{ox} was found in
255 agricultural soils than in semi-natural soils, except for one cropland site (#6) with a low value of
256 $5 \text{ mmol kg}^{-1} P_{ox}$ which was likely associated with its low OC ($0.64 \text{ g } 100\text{g}^{-1}$). Among all sites,
257 site #15, which had the lowest OC content, also had the lowest P_{ox} concentration ($<1.0 \text{ mmol kg}^{-1}$).
258 The national grid sampling in Denmark in 1987 and 1998 showed that agricultural soils
259 contained around twice more P_{ox} and total P than deciduous forest soils at the top 25 cm (Rubæk
260 et al., 2013). In this study, agricultural soils had around four times more P_{ox} than semi-natural
261 soils, which might be due to the accumulation of phosphorus since the last investigation in 1998.

262 *Tea Bag Index*

263 Grasslands and forests are known to store more C (Wiesmeier et al., 2012; Poelau and Don,
264 2013). Thus, higher C stabilization was expected in grasslands and forests. The range of k was
265 from 0.01 to 0.041 and S was from 0.15 to 0.37 for all investigated sites, which were within the
266 range of global TBI investigation (approx. 0.005-0.04 for k and 0.05-0.55 for S) as presented by
267 Keuskamp et al. (2013) (Fig. 2). The average k and S values of agricultural soils were
268 significantly higher than those of semi-natural soils ($p < 0.05$). Among the four land uses,
269 grasslands tend to have the highest values of k and S (Fig. 2); averaging 0.029 ± 0.004 and $0.35 \pm$
270 0.01 , respectively.

271 The average k values of agricultural soils were 0.026 ± 0.002 , while that of semi-natural
272 soils was 0.018 ± 0.001 . The range of k for agricultural soils (0.006 – 0.055) was larger than that
273 for semi-natural soils (0.011 – 0.034). The S seemed to differ with land use (Fig. 2). In partial
274 agreement with our hypothesis, the S of grasslands was higher than that of croplands, while
275 forests exhibited the opposite trend. The highest k and the lowest S in croplands were found at
276 site #4, which had continuous winter wheat monoculture for at least five years, suggesting the
277 adverse effect of continuous monoculture on carbon stabilization as noted previously by
278 Kravchenko et al. (2019). When the land uses were grouped, agricultural soils had an average S
279 value of 0.32 ± 0.008 , and 0.21 ± 0.01 for semi-natural soils. In semi-natural sites, site #20 had
280 the lowest k although it also had the lowest OC among all forest sites. Site #15, with the lowest
281 OC in all sites, had the lowest S . Although there was a little variance in precipitation across the
282 transect of the sites, there is no clear difference in k and S between sites in less rainy area and the
283 wetter area.

284 In agreement with our results, Petraglia et al. (2019) showed that grassland had a higher S
285 (0.3 to 0.4) than shrublands and coniferous forests (0.2 to 0.3) at 10 °C. Higher TBI values in
286 agricultural soils compared to semi-natural soils may be attributed to the fertilization from
287 agricultural management. Thus, there was less nutrient limitation for microbial activity and more
288 compounds might be synthesized and stabilized in the studied three months (Duddigan et al.,
289 2020). For example, croplands treated with organic-mineral fertilizer had higher k (0.343) than
290 unfertilized (0.247) loamy silt soil in Austria (Spiegel et al., 2018). Nevertheless, the impact of
291 land-use intensification on k and S seems to vary. Intensified land use increased k and decreased
292 S along the elevation gradient of Mt. Kilimanjaro (Becker and Kuzyakov, 2018). The k was
293 found higher in croplands than in grasslands in a study in Germany (Yin et al., 2019). On the
294 contrary, Jernej et al. (2019) found that k was lower in managed meadows than abandoned
295 meadows. Another study showed that soil compaction (higher bulk density) in the arable land
296 could result in slower litter decomposition and higher remaining litter mass compared to the less
297 compacted grassland (Carlesso et al., 2019). To further understand the trends observed in our
298 data, the TBI parameters can be linked with soil compaction/bulk density and chemical
299 properties, such as nutrient availability.

300

301 *The relationship between TBI and soil physicochemical properties*

302 The attempt to correlate TBI with soil properties such as clay content, OC, pH, EC, ρ_b , Al_{ox} , Fe_{ox}
303 and P_{ox} proved to be inadequate (Fig. 3). Weak or no correlations were found between TBI and
304 eight soil physicochemical properties, pH being the best relation (Fig. 3 & Table S1). Soil texture
305 had no noticeable effect on TBI. This is probably due to the relatively small variation of soil
306 texture in our samples. Likewise, bulk density solely did not seem to impact TBI. However, there
307 was a positive correlation between pH and S , while no such relationship was found for k (Fig. 3b

308 & d). A similar positive correlation was also found between P_{ox} and S ($r = 0.66$, $p < 0.01$).
309 DeForest (2019) reported that elevated P and pH slowed the decay of tree leaf litter at the later
310 stage of decomposition which was explained by microbial mining theory. The nutrient addition
311 in agricultural soils may have slowed the microbe mining for nutrients from the litter and thus
312 enhanced the stabilization.

313 In Fig. 3d, a clear separation can be seen between the two land-use groups, namely,
314 agricultural and semi-natural, and agricultural soils with high pH had higher S . Motavalli et al.
315 (1995) reported that low pH reduced the decomposition of added plant residues in tropical forest
316 soils. Malik et al. (2018) demonstrated that carbon use efficiency (CUE) was high at pH higher
317 than 6.2, which means that more C was immobilized than respired and it could lead to the
318 accumulation of SOC at high pH. A different threshold of pH was reported by Jones et al.,
319 (2019), where they found that CUE declined at $pH < 5.5$, which is close to the threshold
320 observed in this study. The low k and S in semi-natural soils may be explained by the low C
321 microbial immobilization which was hampered by low pH. At the low pH, P would be bound to
322 Al and Fe and thus not available for microorganisms.

323

324 *Soil gas transport from four land uses*

325 The ε , D_p/D_0 and k_a are parameters that describe soil aeration and structure and they can be
326 altered by land use. The lowest and highest values of ε , D_p/D_0 and k_a values were expected to be
327 found in croplands and forests, respectively. The D_p/D_0 ranged from 0.0023 to 0.14, k_a from 0.43
328 to 476.74 μm^2 and ε from 0.11 to 0.36 $cm^3 cm^{-3}$ for all land uses (Fig. 4). The cropland soils had
329 lower ε and correspondingly lower D_p/D_0 compared to the grassland soils. Similarly, Kreba et al.

330 (2017) showed that the cropland had lower D_p/D_0 than pasture soils in a silt loam site in
331 Kentucky, US. Since intensive agricultural management reduced the diversity of soil fauna
332 (Tsiafouli et al., 2015), along with the compaction of the soil surface, croplands were thus
333 expected to be less structured. Six out of eight cropland sites had ε between 0.1 and 0.2 $\text{cm}^3 \text{cm}^{-3}$,
334 while other land uses had a broader range of ε from 0 to 0.4 $\text{cm}^3 \text{cm}^{-3}$ at field capacity (Fig. 4a &
335 b), suggesting less connected large pores in cropland.

336 Despite the variations in D_p/D_0 and ε among the different land uses, the relationship
337 between the two variables could be adequately described by the model proposed by Moldrup et
338 al. (2000) (Equation 4). The four semi-natural sites fell on the right side of the fitting line,
339 suggesting that they had a potentially larger fraction of blocked air-filled pores that were not
340 involved in the diffusion process (Fig. 4a). Because of its high sand content and associated low
341 water content, the heathland site #15 had the highest D_p/D_0 and ε among all sites. Although
342 forest soils and grasslands had equally high ε values, the D_p/D_0 of forest soil was lower than that
343 of grassland, suggesting a more tortuous structure of forest soils. Schjønning et al. (2003)
344 reported a $D_p/D_0 = 0.025$ for sandy soil as the threshold for optimum aerobic activity. Six out of
345 eight croplands were under the threshold for the optimum aerobic activity at the field capacity,
346 implying that there was some occurrence of anaerobic conditions with consequent inhibition of
347 aerobic microbial activity.

348 In Fig. 4b, the k_a for croplands was lower than other land uses. Two forest sites (#19 and
349 #20), had lower D_p/D_0 but higher k_a compared to the other three forest sites, implying more
350 continuous gas pathways in these two sites. This trend can be explained by their relatively higher
351 fine particle (clay and silt) content, which helps in the formation of aggregates. Samples from
352 sites #1 and #5 had large variations in k_a , with a coefficient of variation of 0.99 and 0.96,

353 respectively (error bar for #1 was removed in Fig. 4b). It might be because of the high soil
354 biological activity that may have created large tortuous channels. In agreement with our
355 hypothesis, cropland soils had lower ε , D_p/D_0 and k_a values compared to the other land-use types
356 at a matric potential of -10 kPa. A similar trend was also reported by Holthusen et al. (2018)
357 where they found forest/grassland had higher k_a than cropland for sandy loam soil at depth 10 –
358 15 cm. The dense grass roots and decayed root channels may have improved the structure in
359 grasslands. Furthermore, it has been shown that the conversion of forest or pasture lands to
360 cropland decreases the fraction of soil macro-aggregates and increases in micro-aggregates
361 (Spohn and Giani, 2011; Wei et al., 2013), resulting in less continuous pore channels in
362 croplands. In contrast to our presumption, higher values of D_p/D_0 were found in grasslands
363 instead of forest soils. It is likely due to the high root density of grassland soils, leading to better
364 pore connectivity than in forest soils.

365

366 *The relationships between TBI, soil properties and land use*

367 Based on the correlations in the previous section, no single basic soil property was able to
368 accurately explain k and S . Therefore, soil gas transport parameters (D_p/D_0 and k_a) were included
369 to ascertain if these variables can explain the observed variations in k and S across the land uses.
370 To our knowledge, this study is the first attempt to link tea bag index with gas transport
371 properties under different land uses. Better prediction was observed for S than for k ; the basic
372 soil physicochemical properties can explain 69% of S and 44% of k (Table 2 & Fig. 5). Clay
373 content and OC were not strong predictors for neither k nor S , possibly due to the narrow range
374 of clay content (Table 2). Soil aeration and structure affect oxygen transport in the soil matrix,
375 and thus we hypothesized that k would increase with D_p/D_0 and k_a , and S would decrease with

376 these two parameters. Therefore, the inclusion of gas transport parameters was expected to
377 improve the prediction of k and S . Although including gas transport parameters indeed improved
378 the model performance for S , it decreased the ability of the model to explain the observed
379 variability in k (Table 2). The soil properties that best explained the variability in k were in some
380 cases different than S . The common predictive variables for the two best-fitted sets for TBI were
381 P_{ox} , EC, ρ_b and land use. The absence of pH in the best-fitted sets was likely due to the
382 correlation between pH and land use. The P_{ox} was negatively correlated to k but positively
383 correlated to S . This is in contrast to the Pearson correlation between P_{ox} and k (Table S1). P_{ox} is
384 correlated to plant-available P in acidic soil (Guo and Yost, 1999) and previous studies have
385 found that P addition could inhibit litter decomposition in forests (Kelly and Henderson, 1978;
386 Chen et al., 2013). This inhibition could be explained by the microbial mining theory (DeForest,
387 2019), where microorganisms invested less energy in mining for nutrients and thus lowered the
388 decomposition. The rest of the common variables in the best-fitted sets, i.e. EC and ρ_b , showed
389 the same negative trends for k and S . High EC and ρ_b would negatively impact k and S and it is in
390 agreement with previously reported adverse effects of EC and ρ_b on microbial activity (Yuan et
391 al., 2007; Carlesso et al., 2019). There were four more variables in the prediction of S compared
392 to k , i.e. Fe_{ox} , Al_{ox} , D_p/D_0 and k_a . Similar to the opposite trend observed in P_{ox} and k , we noted
393 that although Fe_{ox} and Al_{ox} were negatively correlated to S in the model, each property was
394 positively correlated to S (Table S1). It is reported that iron and aluminum oxides lowered the
395 decomposition rate (Miltner and Zech, 1998). Opposite trends may be due to the weak linear
396 relationships between k and P_{ox} , and S and Fe_{ox} and Al_{ox} . Thus, the correlations were easily
397 affected by other factors with a stronger impact on k and S . It should be noted that higher soil
398 aeration would decrease S as suggested by the model and highly structured soil would increase

399 the S . It may be because the access to oxygen would improve the aerobic microbial activity. In
400 the MLR, the cropland land use was used as the reference category to which the other land uses
401 were compared. The grassland had higher k and S while the semi-natural land uses had smaller k
402 and S compared to the cropland. More macropores tend to be found in natural soils than in
403 managed soil (Or et al., 2021), and therefore semi-natural soils are most likely to be nutrient-
404 limited and oxygen is possibly less restricted (Fig. 4a). Thus, microbial respiration possibly
405 responds less to the structure. High competition of microorganisms in large pores and limited
406 nutrients and water may impede SOM turnover. On the other hand, because of the high
407 availability of nutrients and more homogenous soil structure and smaller pores in agricultural
408 soils, microbial activity is more likely constrained by oxygen supply.

409 The low number of tea bags and selected sites can potentially be an experimental
410 limitation of the work. We, therefore, suggest that future investigations include a larger number
411 of tea bags per site, and also include experimental measures of microbial activity and diversity.

412

413

CONCLUSION

414 The study investigated linkages between litter decomposition, quantified by the Tea Bag Index
415 (TBI) parameters (S and k), and soil physicochemical properties and gas transport under different
416 land uses. Basic soil physicochemical properties (pH, EC, ρ_b and P_{ox}) could better predict the
417 capacity of soil to stabilize OM (S) than the capacity to decompose OM (k), and the inclusion of
418 land use improved the prediction accuracy for both TBI parameters. However, clay content and
419 OC were not strong predictors for the TBI and there seemed to be no effect of soil gas transport
420 on k . The results confirmed our hypothesis that litter decomposition exhibited differently across
421 the four land uses (cropland, grassland, heath and forest). In contrast to our hypothesis,

422 agricultural soils tended to have higher k and S than semi-natural soils. Grassland had a higher
423 ability to decompose labile SOM and potentially stabilize C than the other land uses in this
424 study. The pH, nutrient availability and the compaction of soil may be the main reasons
425 contributing to the difference in litter decomposition between the four land uses.

426

427

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Figure 1. Location of the investigated sites.

Figure 2. Tea Bag Index parameters for the investigated sites differentiated according to prevailing land use.

Figure 3. Relationships between the Tea Bag Index parameters (k & S) and (a,c) bulk density, and (b, d) soil pH. The r indicates the Pearson correlation coefficient. *ns*: not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. The error bars indicate the standard error of the mean.

Figure 4. The relationship between air-filled porosity and (a) relative gas diffusivity (D_p/D_0), and (b) air permeability (k_a) at a matric potential of -10 kPa for the four land uses. The numbers in the plots are the site IDs. The grey dashed line in (a) is the Moldrup et al. (2000) model $\frac{D_p,10}{D_0} = 2\varepsilon_{10}^3 + 0.04\varepsilon_{10}$. The dotted line in (a) is the D_p/D_0 for optimum aerobic microbial activity (Schjønning et al., 2003). The error bars indicate the standard error of the mean. Due to the large variation in k_a , the error bar of site #1 in (b) is not shown.

Figure 5. Scatterplots of predicted versus measured (a, c, e) decomposition rate k and (b, d, f) stabilization factor S with multiple linear regression models using one, two and three sets of variables. The inputs for the models include selected physicochemical properties in panels a and b, selected physicochemical properties and gas transport parameters in c and d, and selected physicochemical properties, gas transport parameters and land use in e and f. The error bars indicate the standard error of the mean.

619 **Table 1.** Land use, vegetation and soil physicochemical properties for the investigated sites. The numbers in the parentheses under
 620 the agricultural land use are the fertilization rate (mineral fertilizer/organic fertilizer, kg N ha⁻¹).

Site ID	Land use	Dominant vegetation	Clay	Silt g 100g ⁻¹	Sand	OC ^a	pH (H ₂ O)	EC mS cm ⁻¹	ρ_b g cm ⁻³	Al _{ox}	Fe _{ox} mmol kg ⁻¹	P _{ox}
Agricultural												
1	Cropland (64/131)	Winter hybrid rye	5	13	77	2.49	5.3	0.65	1.20	87	53	22
2	Cropland (95/97)	Winter hybrid rye	7	10	79	1.74	5.6	0.48	1.40	29	350	31
3	Cropland (140/23)	Winter wheat	7	28	61	2.38	5.6	0.56	1.35	89	54	28
4	Cropland (104/0)	Winter wheat	10	26	60	2.15	5.9	0.56	1.37	79	56	25
5	Cropland (101/83)	Winter wheat	14	24	59	1.68	6.2	0.55	1.40	44	59	22
6	Cropland (106/95)	Winter barley	9	12	78	0.64	6.5	0.44	1.62	26	29	5.0
7	Cropland (70/103)	Winter wheat	6	11	81	1.16	6.4	0.65	1.38	48	41	28
8	Cropland (145/33)	Winter wheat	35	38	12	7.25	7.7	2.16	0.64	26	210	18
9	Grassland (0/0)	Perennial grass	4	5	89	1.16	5.7	0.36	1.37	41	32	15
10	Grassland (0/0)	Grass with < 50% clover	3	7	86	2.26	5.7	0.27	1.25	76	73	14
11	Grassland (189/94)	Grass without clover	7	7	83	1.57	6.2	0.57	1.22	35	48	18
12	Grassland (0/63)	Grass with < 50% clover	7	12	77	2.20	7.7	1.13	1.44	26	44	27
Semi-natural												
13	Heath	Heather	3	9	83	2.03	4.6	0.19	1.33	38	48	4.2
14	Heath	Heather	3	9	83	2.26	5.0	0.30	1.37	41	44	3.1
15	Heath	Heather	2	2	96	0.23	5.3	0.07	1.58	3	6.5	<1.0

16	Forest	Scots pine	5	12	79	2.03	4.3	0.30	1.23	38	41	7.6
17	Forest	Scots pine	1	7	88	2.15	4.7	0.21	1.29	7.5	9.7	1.1
18	Forest	Heather/ European beech	5	7	83	2.38	4.6	0.66	1.14	24	65	9.4
19	Forest	European beech	13	25	59	2.09	4.8	0.65	1.13	34	45	6.1
20	Forest	European beech	11	25	61	1.68	4.5	0.40	1.25	32	46	5.8

621 ^aOC, organic carbon; EC, electrical conductivity; ρ_b , bulk density; Al_{ox}, Fe_{ox}, P_{ox} are oxalate-extractable aluminum, iron and
622 phosphorus, respectively.

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624 **Table 2.** Prediction of decomposition rate (k) and stabilization factor (S) with multiple linear regression analysis (MLR). The adjusted
625 R² of MLR with selected one, two and three sets of variables are presented in the first three rows. The “–” indicates that the parameter
626 is negatively correlated to k or S . The “+” indicates a positive correlation. *Basics* include eight basic soil physicochemical properties.
627 *Gas transport* includes relative gas diffusivity (D_p/D_0) and air permeability (k_a). *Land use* refers to the categories of cropland,
628 grassland, forest and heath. The cropland is the baseline category for MLR.

Input parameters for MLR	k		S	
	Selected variables	R ²	Selected variables	R ²
Basics	pH, EC, ρ_b	0.44**	pH, P _{ox} , EC, ρ_b	0.69***
Basics, Gas transport	pH, EC, ρ_b , D_p/D_0 , k_a	0.40*	pH, P _{ox} , Fe _{ox} , Al _{ox} , EC, ρ_b , D_p/D_0 , k_a	0.83***
Basics, Gas transport, Land use	P _{ox} , EC, ρ_b , Land use	0.63**	P _{ox} , Fe _{ox} , Al _{ox} , EC, ρ_b , D_p/D_0 , k_a , Land use	0.86***
Variables from the best-fit set				
	P _{ox}	–	P _{ox}	+
Basics			Fe _{ox}	–
			Al _{ox}	–
	EC	–	EC	–
Gas transport	ρ_b	–	ρ_b	–
			k_a	+
			D_p/D_0	–
Land use	Grassland	+	Grassland	+
	Forest	–	Forest	–

Heath

–

Heath

–

629 Al_{ox} , Fe_{ox} , P_{ox} are oxalate-extractable aluminum, iron and phosphorus, respectively; EC, electrical conductivity; ρ_{b} , bulk density.
630 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.