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## Linking litter decomposition to soil physicochemical properties, gas transport, and land use

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*Published in:*  
Soil Science Society of America Journal

*DOI (link to publication from Publisher):*  
[10.1002/saj2.20356](https://doi.org/10.1002/saj2.20356)

*Publication date:*  
2022

*Document Version*  
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*

Fu, Y., de Jonge, L. W., Greve, M. H., Arthur, E., Moldrup, P., Norgaard, T., & Paradelo, M. (2022). Linking litter decomposition to soil physicochemical properties, gas transport, and land use. *Soil Science Society of America Journal*, 86(1), 34-46. <https://doi.org/10.1002/saj2.20356>

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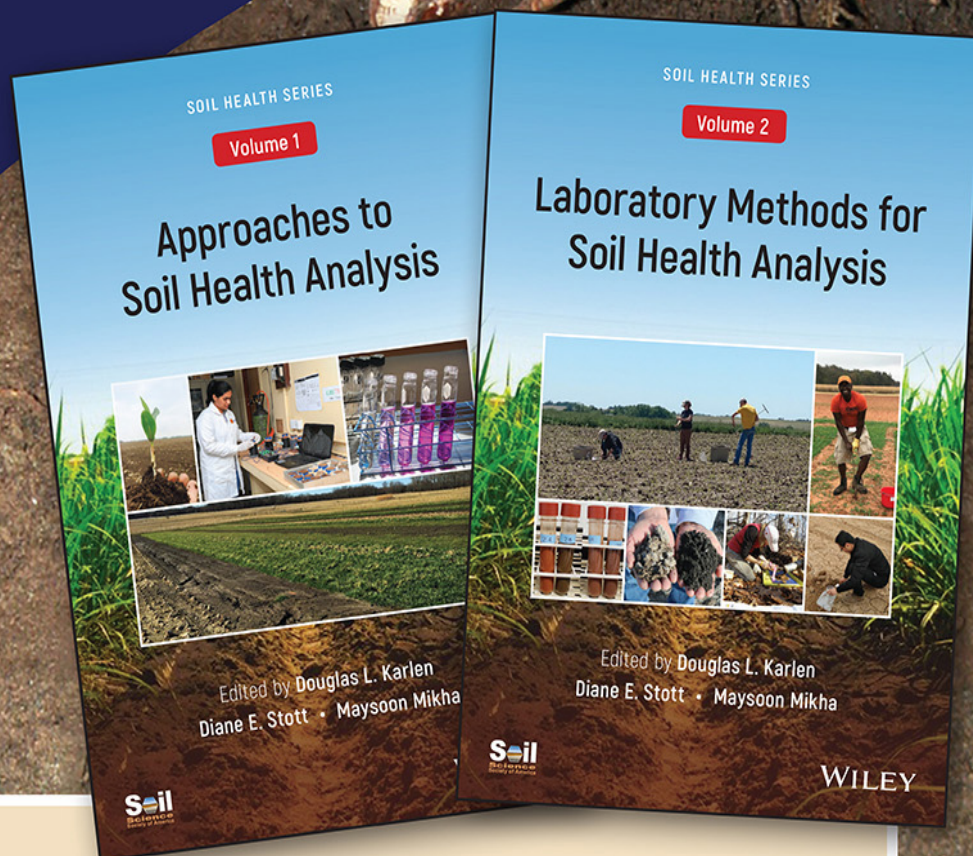
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Running title *Linking Tea Bag Index to soil properties and land use*

### Core ideas

- Litter decomposition, quantified by the Tea Bag Index (TBI), was determined across land uses
- Grasslands had the highest decomposition rate and stabilization factor of plant litter
- Soil gas transport was more important to stabilization factor, not decomposition rate
- Variability in the TBI parameters was affected by soil pH, P<sub>oxalate</sub> and bulk density
- The inclusion of land use improved the predictions of both TBI parameters

### Linking Litter Decomposition to Soil Physicochemical Properties, Gas Transport and Land Use

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Abbreviations:  $Al_{ox}$ , oxalate-extractable aluminum;  $D_0$ , oxygen diffusion in free air;  $D_p$ , oxygen diffusion in soil;  $D_p/D_0$ , relative gas diffusivity;  $\varepsilon$ , air-filled porosity; EC, electrical conductivity;  $Fe_{ox}$ , oxalate-extractable iron;  $k$ , decomposition rate;  $k_a$ , air permeability;  $\phi$ , total porosity;  $P_{ox}$ , oxalate-extractable phosphorus;  $\rho_b$ , bulk density;  $\rho_s$ , particle density; RMSE, root mean square error;  $S$ , stabilization factor; SOC, soil organic carbon; SOM, soil organic matter; TBI, Tea Bag Index.

### ABSTRACT

Litter decomposition is a critical process in carbon cycling, which can be impacted by land use. The relationship between litter decomposition and soil properties under different land uses remains unclear. Litter decomposition can be quantified by the Tea Bag Index (TBI), which includes a decomposition rate  $k$ , and a stabilization factor  $S$ . Our objective was to investigate linkages between TBI and soil physicochemical and gas transport properties and land use. We buried three pairs of tea bags in 20 sites (covering cropland, grassland, heathland, and forest land uses) in a transect from the western to the eastern coast of the Jutland peninsula, Denmark. The tea bags were retrieved after 90 days and TBI was determined. Disturbed and undisturbed ( $100\text{ cm}^3$  soil cores) samples were collected from each site. Thereafter, clay content, organic carbon, bulk density, pH, electrical conductivity, oxalate-extractable phosphorus ( $P_{ox}$ ), aluminum and iron content, soil water content, gas diffusivity ( $D_p/D_0$ ) and air permeability ( $k_a$ ) at  $-10\text{ kPa}$  were measured. Results showed that grasslands had the highest  $k$  and  $S$  among four land uses, and agricultural soils (croplands and grasslands) exhibited higher TBI values than semi-natural soils (forest and heathland). The prediction of  $S$  was better than that of  $k$  based on multiple linear regression analysis involving

soil physicochemical properties. Clay content and organic carbon were not strong predictors. Including  $D_p/D_0$  and  $k_a$  improved the prediction of  $S$ , and finally, the inclusion of land use enhanced the prediction of both  $k$  and  $S$ . The different trends between two distinct land-use groups can be attributed to pH,  $P_{ox}$  and bulk density.

Keywords: Tea Bag Index, Soil gas diffusivity, Soil air permeability

## INTRODUCTION

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

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

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

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

seen as a way to promote C storage, the amount of C retained in grassland is also strongly dependent on management (Yeasmin et al., 2020).

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

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



land uses, 2) to quantify the litter decomposition and stabilization of different land uses, and 3) to analyze the effect of soil properties, soil gas transport and land use on the TBI  $k$  and  $S$  parameters.

## MATERIALS AND METHODS

### Description of study sites

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

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

Site #7 received the least amount of NPK fertilizer among all sites. The rotation elements in the cropland were common cereals including the aforementioned winter cereals, spring barley and spring oats. Site #8 had winter rape grown in one of the past five years and spinach in one year. Site #4 had winter wheat grown for five consecutive years. The grasslands were kept for grazing and fertilized with manure and/or mineral fertilizer, except for the one with clover which received no fertilizer. The heathlands were dominated by heather (*Calluna vulgaris*). In forests, Scots pine (*Pinus sylvestris*), birch (*Betula pubescens/Betula pendula*), willow (*Salicaceae*) and European beech (*Fagus sylvatica*) were the dominant species. Based on the extent of human activities involved in these lands, we grouped the four land uses into two groups, agricultural (cropland and grassland) and semi-natural (heathland and forest) groups. Detailed information on each site is listed in Table 1.

### Tea Bag Index

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

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

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

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

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

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

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

### Soil sampling and analysis

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

by Schoumans (2000). Soil pH was measured using a pH meter (PHM220, Radiometer Analytical SAS, Lyon) in a soil suspension of 8 cm<sup>3</sup> soil in 30 mL deionized water. Soil EC was measured using an EC meter (CDM210, Radiometer Analytical SAS, Lyon) in a soil suspension of 4 g soil in 36 mL deionized water.

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

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

After the gas transport measurements, the soil cores were oven-dried at 105°C for 24 h to determine the oven-dried mass. The bulk density ( $\rho_b$ ) was estimated as the ratio of oven-dried mass to the total volume of the soil core. The soil particle density ( $\rho_s$ ) was estimated based on the clay and SOM content using the model given by Schjønning et al. (2017). The

total porosity ( $\phi$ ,  $\text{m}^3 \text{m}^{-3}$ ) was calculated from  $\rho_b$  and  $\rho_s$ . The air-filled porosity ( $\varepsilon$ ,  $\text{m}^3 \text{m}^{-3}$ ) was calculated from  $\phi$  and volumetric water content.

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

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

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

### Statistical analyses

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

Before the statistical analyses, normality and equal variance of the data were evaluated using the Shapiro Wilk and Bartlett's tests, respectively. The Pearson correlation coefficient was used to analyze the correlation between TBI parameters and basic soil properties. The criterion of significance was  $p < 0.05$ . Multiple linear regression (MLR) was conducted to analyze the effect of physicochemical soil properties, gas transport parameters and land use on  $k$  and  $S$ . The variables for the MLR were selected by stepwise regression from both directions using the "MASS" package. The first subset of variables were eight soil properties, including clay, OC, pH, EC, bulk density,  $\text{Al}_{\text{ox}}$ ,  $\text{Fe}_{\text{ox}}$ ,  $\text{P}_{\text{ox}}$ . The second subset included the first subset and two gas transport parameters, i.e.  $k_a$  and  $D_p/D_0$ , and the third subset added land use to the second subset. The selection of variables was based on AIC scores and the adjusted  $R^2$  of MLR. The final model from each subset contained the least number of variables with the best prediction. To evaluate the fitting models with the measured data, the RMSE was used for  $n=20$  data points:

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

## RESULTS AND DISCUSSION

Among the 20 sites, 19 sites had sandy soil with clay content ranging from 1 to 14 g 100g<sup>-1</sup> (Table 1). The average OC content for these 19 samples was 1.80 ± 0.14 g 100g<sup>-1</sup>. One cropland site (#8) was peat soil and it had the highest clay content and high OC content, with a resultant very low bulk density of 0.64 g cm<sup>-3</sup>. Site #15 was a heathland on the west coast, and it had the lowest OC and the highest sand content among all sites. Despite the small differences in the soil texture between sites (except for #8), heathlands had the highest average bulk density (1.43 ± 0.04 g cm<sup>-3</sup>), followed by croplands (1.39 ± 0.03 g cm<sup>-3</sup>), grasslands (1.32 ± 0.03 g cm<sup>-3</sup>), and forests (1.20 ± 0.02 g cm<sup>-3</sup>). The peat site #8 was excluded from the calculation of the bulk density averages due to its very low bulk density. A similar trend was found in two German sites, where the topsoil of croplands had higher bulk density than grasslands and forest, and forest soils had the lowest bulk density (Bormann and Klaassen, 2008). The high bulk density of heathland was because of the high sand content. Based on the similarity in soil management among the four land uses, cropland and grassland were grouped as agricultural soils, while heathland and forest were grouped as semi-natural soils. Agricultural soils were less acidic (pH~6.2) than the semi-natural soils (pH~4.6) (Table 1). Several studies also found forest soils had lower pH than agricultural soils (e.g. Falkengren-Grerup et al., 2006; Wiesmeier et al., 2012), which may be explained by the leaching and the lack of calcium input in forest soils (Yesilonis et al., 2016). Agricultural

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practices such as liming practice are often used in the acidic agricultural soils for securing crop yield, and the manure addition has a liming effect on acidic soils (Ozlu and Kumar, 2018). Agricultural soils had higher EC than the semi-natural land uses, except for two outliers in grassland and forest, with average values of  $0.70 \pm 0.14$  and  $0.35 \pm 0.07$  mS cm<sup>-1</sup>, respectively. Ozlu & Kumar (2018) reported that fields with manure input had higher EC than mineral fertilizer and control treatments, which can be attributed to the dissolved salts in manure (Azeez and van Averbek, 2012). Likewise, due to fertilization, higher P<sub>ox</sub> was found in agricultural soils than in semi-natural soils, except for one cropland site (#6) with a low value of 5 mmol kg<sup>-1</sup> P<sub>ox</sub> which was likely associated with its low OC (0.64 g 100g<sup>-1</sup>). Among all sites, site #15, which had the lowest OC content, also had the lowest P<sub>ox</sub> concentration (<1.0 mmol kg<sup>-1</sup>). The national grid sampling in Denmark in 1987 and 1998 showed that agricultural soils contained around twice more P<sub>ox</sub> and total P than deciduous forest soils at the top 25 cm (Rubæk et al., 2013). In this study, agricultural soils had around four times more P<sub>ox</sub> than semi-natural soils, which might be due to the accumulation of phosphorus since the last investigation in 1998.



## Tea Bag Index

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

The average  $k$  values of agricultural soils were  $0.026 \pm 0.002$ , while that of semi-natural soils was  $0.018 \pm 0.001$ . The range of  $k$  for agricultural soils (0.006 – 0.055) was larger than that for semi-natural soils (0.011 – 0.034). The  $S$  seemed to differ with land use (Fig. 2). In partial agreement with our hypothesis, the  $S$  of grasslands was higher than that of croplands, while forests exhibited the opposite trend. The highest  $k$  and the lowest  $S$  in croplands were found at site #4, which had continuous winter wheat monoculture for at least five years, suggesting the adverse effect of continuous monoculture on carbon stabilization as noted previously by Kravchenko et al. (2019). When the land uses were grouped, agricultural soils had an average  $S$  value of  $0.32 \pm 0.008$ , and  $0.21 \pm 0.01$  for semi-natural soils. In semi-

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natural sites, site #20 had the lowest  $k$  although it also had the lowest OC among all forest sites. Site #15, with the lowest OC in all sites, had the lowest  $S$ . Although there was a little variance in precipitation across the transect of the sites, there is no clear difference in  $k$  and  $S$  between sites in less rainy area and the wetter area.

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

### The relationship between TBI and soil physicochemical properties

The attempt to correlate TBI with soil properties such as clay content, OC, pH, EC,  $\rho_b$ ,  $Al_{ox}$ ,  $Fe_{ox}$  and  $P_{ox}$  proved to be inadequate (Fig. 3). Weak or no correlations were found between TBI and eight soil physicochemical properties, pH being the best relation (Fig. 3 & Table S1). Soil texture had no noticeable effect on TBI. This is probably due to the relatively small variation of soil texture in our samples. Likewise, bulk density solely did not seem to impact TBI. However, there was a positive correlation between pH and  $S$ , while no such relationship was found for  $k$  (Fig. 3b & d). A similar positive correlation was also found between  $P_{ox}$  and  $S$  ( $r = 0.66$ ,  $p < 0.01$ ). DeForest (2019) reported that elevated P and pH slowed the decay of tree leaf litter at the later stage of decomposition which was explained by microbial mining theory. The nutrient addition in agricultural soils may have slowed the microbe mining for nutrients from the litter and thus enhanced the stabilization.

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

## Soil gas transport from four land uses

The  $\varepsilon$ ,  $D_p/D_0$  and  $k_a$  are parameters that describe soil aeration and structure and they can be altered by land use. The lowest and highest values of  $\varepsilon$ ,  $D_p/D_0$  and  $k_a$  values were expected to be found in croplands and forests, respectively. The  $D_p/D_0$  ranged from 0.0023 to 0.14,  $k_a$  from 0.43 to 476.74  $\mu\text{m}^2$  and  $\varepsilon$  from 0.11 to 0.36  $\text{cm}^3 \text{cm}^{-3}$  for all land uses (Fig. 4). The cropland soils had lower  $\varepsilon$  and correspondingly lower  $D_p/D_0$  compared to the grassland soils. Similarly, Kreba et al. (2017) showed that the cropland had lower  $D_p/D_0$  than pasture soils in a silt loam site in Kentucky, US. Since intensive agricultural management reduced the diversity of soil fauna (Tsiafouli et al., 2015), along with the compaction of the soil surface, croplands were thus expected to be less structured. Six out of eight cropland sites had  $\varepsilon$  between 0.1 and 0.2  $\text{cm}^3 \text{cm}^{-3}$ , while other land uses had a broader range of  $\varepsilon$  from 0 to 0.4  $\text{cm}^3 \text{cm}^{-3}$  at field capacity (Fig. 4a & b), suggesting less connected large pores in cropland.

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

In Fig. 4b, the  $k_a$  for croplands was lower than other land uses. Two forest sites (#19 and #20), had lower  $D_p/D_0$  but higher  $k_a$  compared to the other three forest sites, implying more continuous gas pathways in these two sites. This trend can be explained by their relatively higher fine particle (clay and silt) content, which helps in the formation of aggregates. Samples from sites #1 and #5 had large variations in  $k_a$ , with a coefficient of variation of 0.99 and 0.96, respectively (error bar for #1 was removed in Fig. 4b). It might be because of the high soil biological activity that may have created large tortuous channels. In agreement with our hypothesis, cropland soils had lower  $\epsilon$ ,  $D_p/D_0$  and  $k_a$  values compared to the other land-use types at a matric potential of  $-10$  kPa. A similar trend was also reported by Holthusen et al. (2018) where they found forest/grassland had higher  $k_a$  than cropland for sandy loam soil at depth 10 – 15 cm. The dense grass roots and decayed root channels may have improved the structure in grasslands. Furthermore, it has been shown that the conversion of forest or pasture lands to cropland decreases the fraction of soil macro-aggregates and increases in micro-aggregates (Spohn and Giani, 2011; Wei et al., 2013), resulting in less continuous pore channels in croplands. In contrast to our presumption, higher values of  $D_p/D_0$  were found in grasslands instead of forest soils. It is likely due to the high root density of grassland soils, leading to better pore connectivity than in forest soils.

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

Based on the correlations in the previous section, no single basic soil property was able to accurately explain  $k$  and  $S$ . Therefore, soil gas transport parameters ( $D_p/D_0$  and  $k_a$ ) were included to ascertain if these variables can explain the observed variations in  $k$  and  $S$  across the land uses. To our knowledge, this study is the first attempt to link tea bag index with gas transport properties under different land uses. Better prediction was observed for  $S$  than for  $k$ ;

the basic soil physicochemical properties can explain 69% of  $S$  and 44% of  $k$  (Table 2 & Fig. 5). Clay content and OC were not strong predictors for neither  $k$  nor  $S$ , possibly due to the narrow range of clay content (Table 2). Soil aeration and structure affect oxygen transport in the soil matrix, and thus we hypothesized that  $k$  would increase with  $D_p/D_0$  and  $k_a$ , and  $S$  would decrease with these two parameters. Therefore, the inclusion of gas transport parameters was expected to improve the prediction of  $k$  and  $S$ . Although including gas transport parameters indeed improved the model performance for  $S$ , it decreased the ability of the model to explain the observed variability in  $k$  (Table 2). The soil properties that best explained the variability in  $k$  were in some cases different than  $S$ . The common predictive variables for the two best-fitted sets for TBI were  $P_{ox}$ , EC,  $\rho_b$  and land use. The absence of pH in the best-fitted sets was likely due to the correlation between pH and land use. The  $P_{ox}$  was negatively correlated to  $k$  but positively correlated to  $S$ . This is in contrast to the Pearson correlation between  $P_{ox}$  and  $k$  (Table S1).  $P_{ox}$  is correlated to plant-available P in acidic soil (Guo and Yost, 1999) and previous studies have found that P addition could inhibit litter decomposition in forests (Kelly and Henderson, 1978; Chen et al., 2013). This inhibition could be explained by the microbial mining theory (DeForest, 2019), where microorganisms invested less energy in mining for nutrients and thus lowered the decomposition. The rest of the common variables in the best-fitted sets, i.e. EC and  $\rho_b$ , showed the same negative trends for  $k$  and  $S$ . High EC and  $\rho_b$  would negatively impact  $k$  and  $S$  and it is in agreement with previously reported adverse effects of EC and  $\rho_b$  on microbial activity (Yuan et al., 2007; Carlesso et al., 2019). There were four more variables in the prediction of  $S$  compared 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 that although  $Fe_{ox}$  and  $Al_{ox}$  were negatively correlated to  $S$  in the model, each property was positively correlated to  $S$  (Table S1). It is reported that iron and aluminum oxides lowered the

decomposition rate (Miltner and Zech, 1998). Opposite trends may be due to the weak linear relationships between  $k$  and  $P_{ox}$ , and  $S$  and  $Fe_{ox}$  and  $Al_{ox}$ . Thus, the correlations were easily affected by other factors with a stronger impact on  $k$  and  $S$ . It should be noted that higher soil aeration would decrease  $S$  as suggested by the model and highly structured soil would increase the  $S$ . It may be because the access to oxygen would improve the aerobic microbial activity. In the MLR, the cropland land use was used as the reference category to which the other land uses were compared. The grassland had higher  $k$  and  $S$  while the semi-natural land uses had smaller  $k$  and  $S$  compared to the cropland. More macropores tend to be found in natural soils than in managed soil (Or et al., 2021), and therefore semi-natural soils are most likely to be nutrient-limited and oxygen is possibly less restricted (Fig. 4a). Thus, microbial respiration possibly responds less to the structure. High competition of microorganisms in large pores and limited nutrients and water may impede SOM turnover. On the other hand, because of the high availability of nutrients and more homogenous soil structure and smaller pores in agricultural soils, microbial activity is more likely constrained by oxygen supply.

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

## CONCLUSION

The study investigated linkages between litter decomposition, quantified by the Tea Bag Index (TBI) parameters ( $S$  and  $k$ ), and soil physicochemical properties and gas transport under different land uses. Basic soil physicochemical properties (pH, EC,  $\rho_b$  and  $P_{ox}$ ) could better predict the capacity of soil to stabilize OM ( $S$ ) than the capacity to decompose OM ( $k$ ),

and the inclusion of land use improved the prediction accuracy for both TBI parameters. However, clay content and OC were not strong predictors for the TBI and there seemed to be no effect of soil gas transport on  $k$ . The results confirmed our hypothesis that litter decomposition exhibited differently across the four land uses (cropland, grassland, heath and forest). In contrast to our hypothesis, agricultural soils tended to have higher  $k$  and  $S$  than semi-natural soils. Grassland had a higher ability to decompose labile SOM and potentially stabilize C than the other land uses in this study. The pH, nutrient availability and the compaction of soil may be the main reasons contributing to the difference in litter decomposition between the four land uses.

#### ACKNOWLEDGEMENTS

The work was supported by the Danish Council for Independent Research, Technology and Production Sciences via the project: Biophysical drivers of soil resilience in a changing climate [grant number 9041-00107B]. MP was funded by the Research England 'Expanding Excellence in England' (E3) – FaNSI programme of the NRI. We especially thank Birger Faurholt Pedersen for providing the information of the investigated sites. The assistance of Stig Rasmussen and Bodil Christensen with sampling and gas transport measurements is appreciated.



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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/saj2.20356](https://doi.org/10.1002/saj2.20356).

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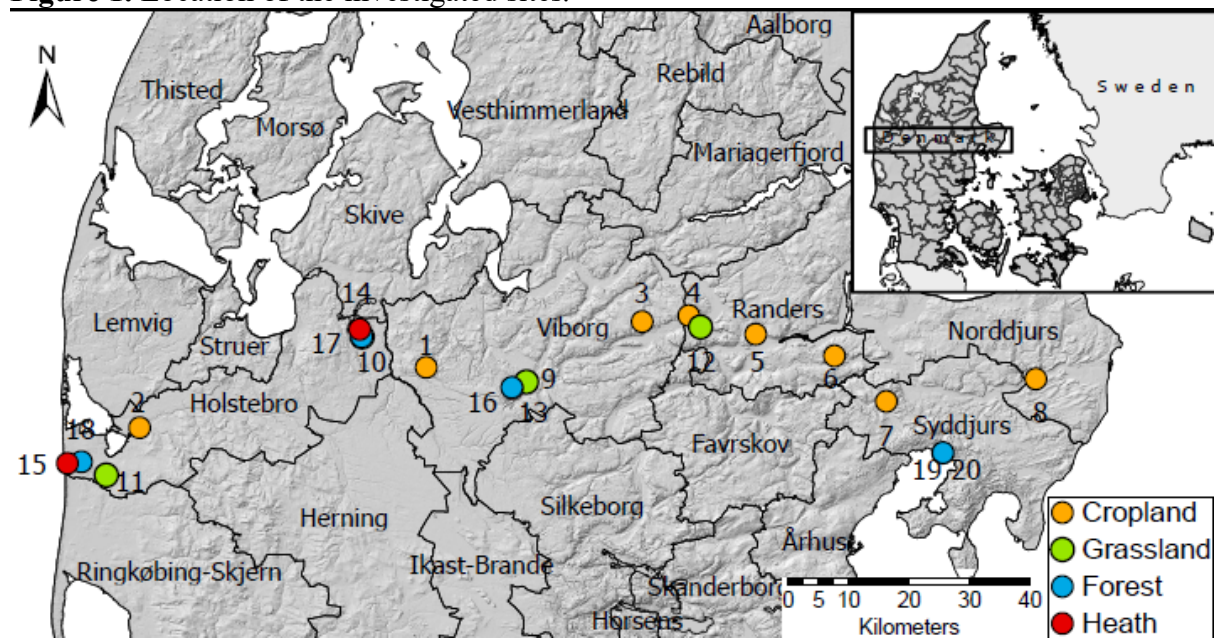
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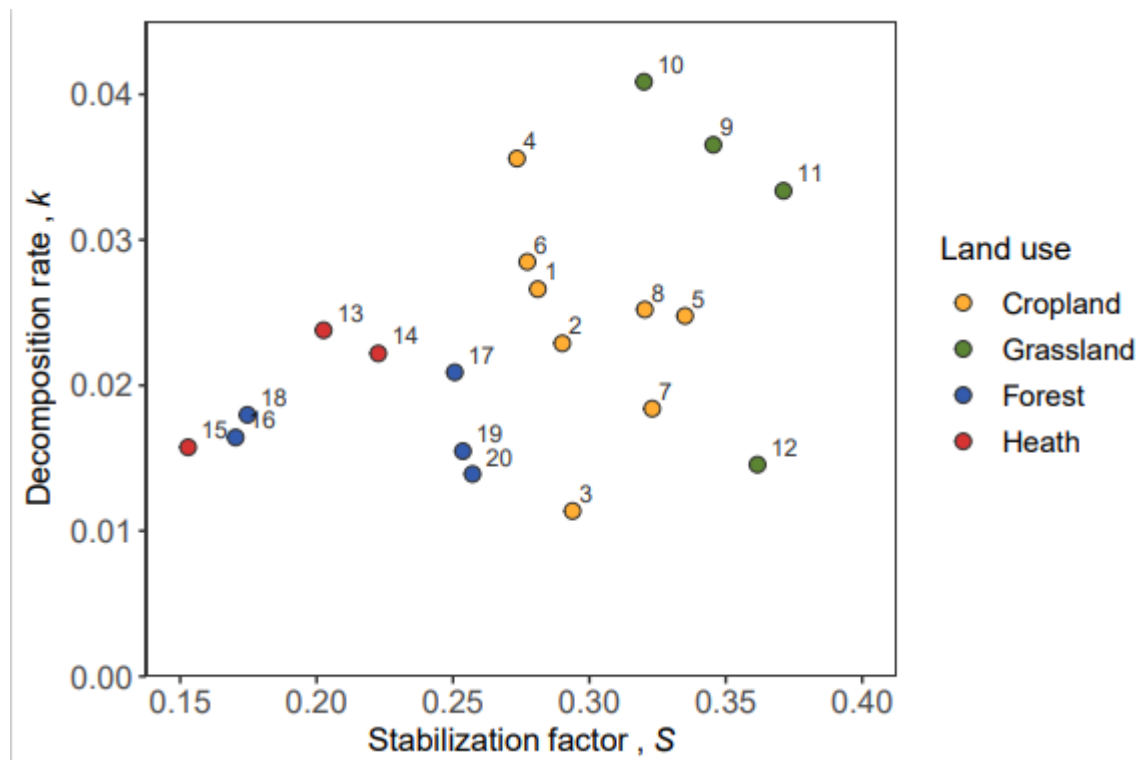
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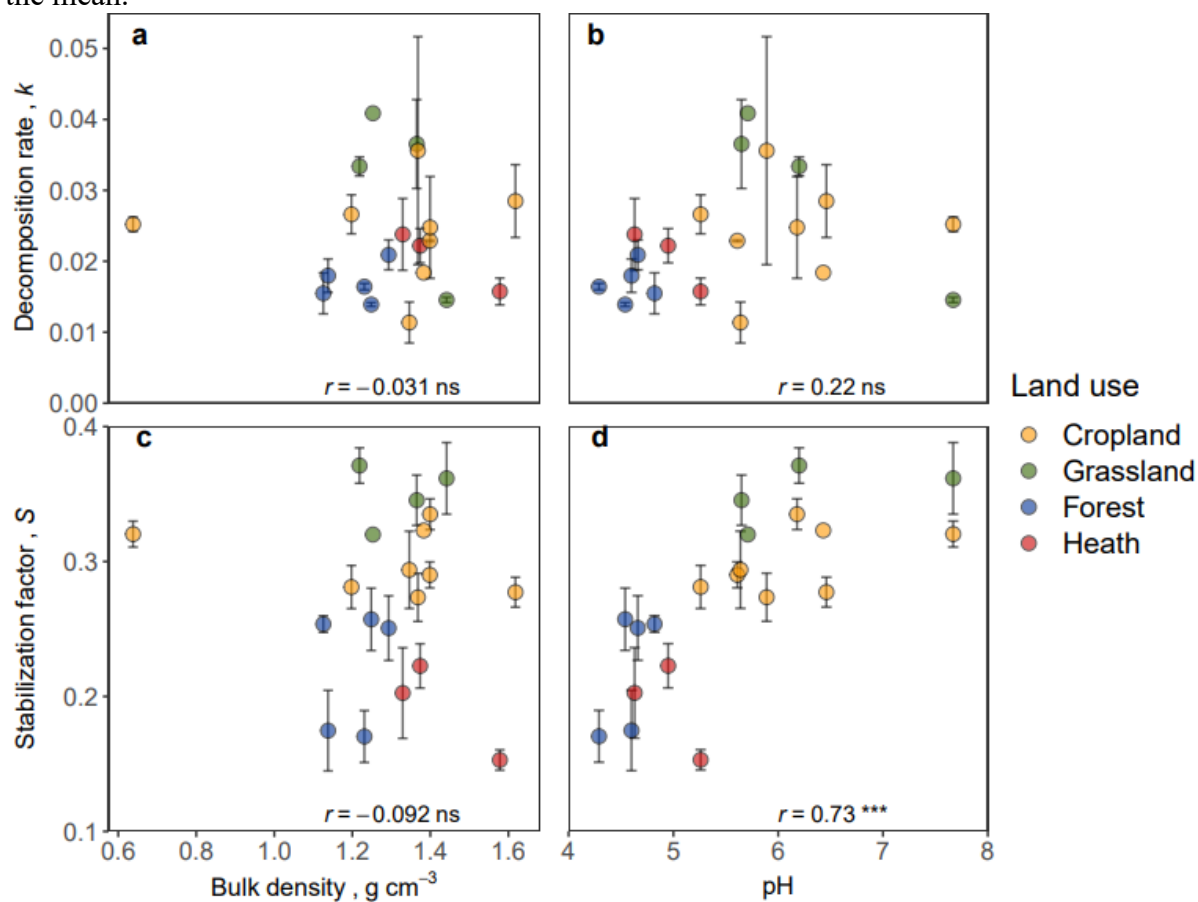
**Figure 1.** Location of the investigated sites.



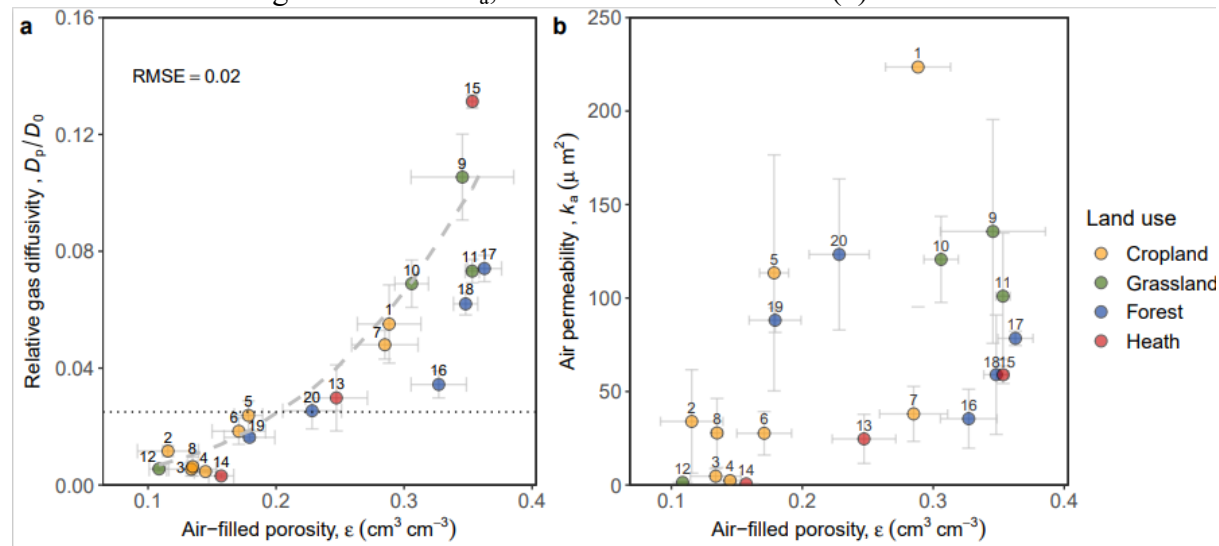
**Figure 2.** Tea Bag Index parameters for the investigated sites differentiated according to prevailing land use.



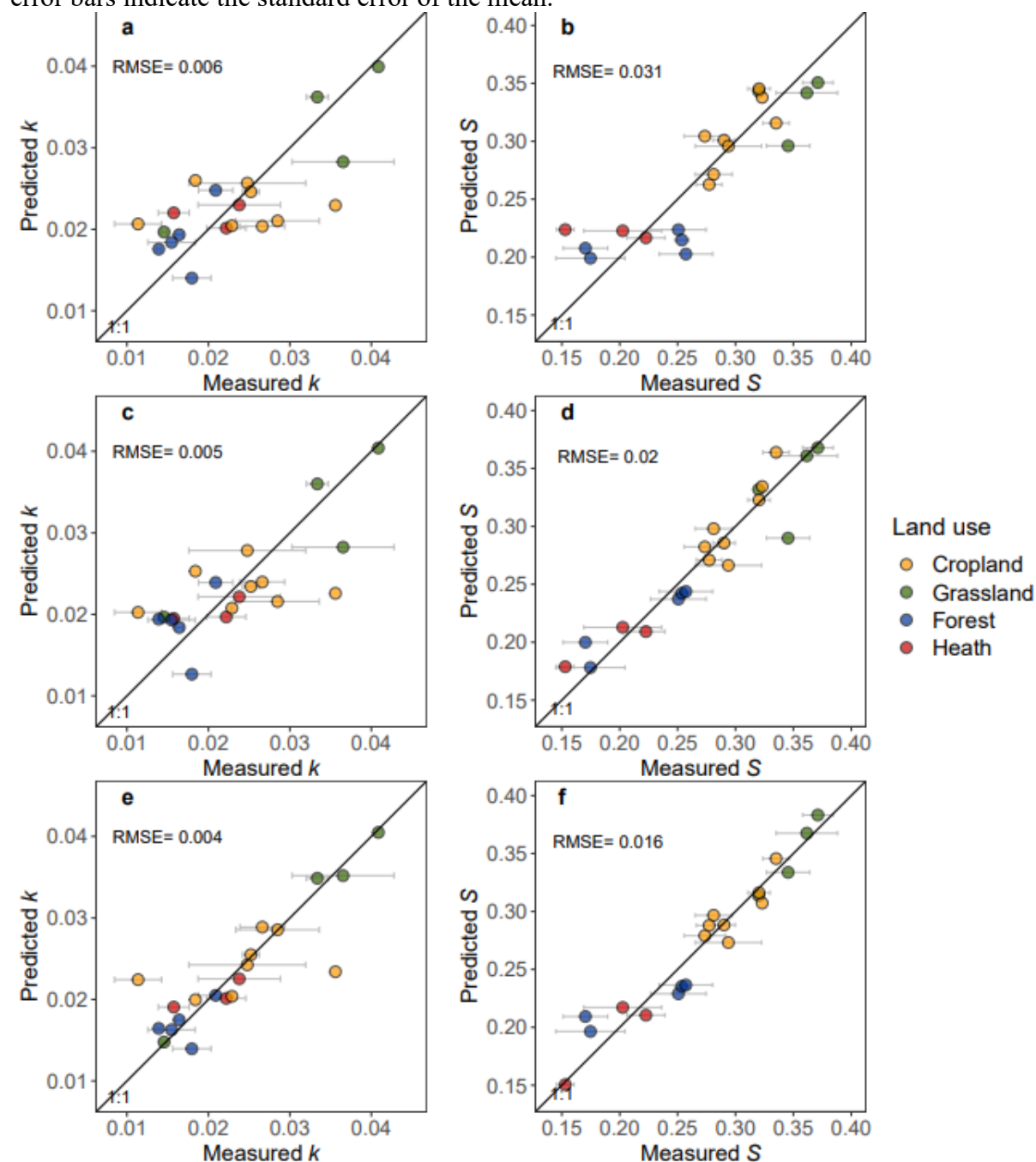
**Figure 3.** Relationships between the Tea Bag Index parameters ( $k$  &  $S$ ) and (a,c) bulk density, and (b, d) soil pH. The  $r$  indicates the Pearson correlation coefficient. *ns*: not significant,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ . The error bars indicate the standard error of the 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  $\frac{D_{p,10}}{D_0} = 2\epsilon_{10}^3 + 0.04\epsilon_{10}$ . 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.



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

Sit e ID	Land use	Dominan t vegetatio n	Clay	Silt	Sand	OC <sup>a</sup>	pH (H <sub>2</sub> O )	EC mS cm <sup>-1</sup>	$\rho_b$ g cm <sup>-3</sup>	Al <sub>o</sub> x	Fe <sub>o</sub> x	P <sub>ox</sub>
					g 100g <sup>-1</sup>						mmol kg <sup>-1</sup>	
<b>Agricultural</b>												
1	Cropland (64/131)	Winter hybrid rye	5	13	77	2.4 9	5.3	0.6 5	1.2 0	87	53	22
2	Cropland (95/97)	Winter hybrid rye	7	10	79	1.7 4	5.6	0.4 8	1.4 0	29	350	31
3	Cropland (140/23)	Winter wheat	7	28	61	2.3 8	5.6	0.5 6	1.3 5	89	54	28
4	Cropland (104/0)	Winter wheat	10	26	60	2.1 5	5.9	0.5 6	1.3 7	79	56	25
5	Cropland (101/83)	Winter wheat	14	24	59	1.6 8	6.2	0.5 5	1.4 0	44	59	22
6	Cropland (106/95)	Winter barley	9	12	78	0.6 4	6.5	0.4 4	1.6 2	26	29	5.0
7	Cropland (70/103)	Winter wheat	6	11	81	1.1 6	6.4	0.6 5	1.3 8	48	41	28
8	Cropland (145/33)	Winter wheat	35	38	12	7.2 5	7.7	2.1 6	0.6 4	26	210	18
9	Grasslan d (0/0)	Perennial grass	4	5	89	1.1 6	5.7	0.3 6	1.3 7	41	32	15
10	Grasslan d (0/0)	Grass with < 50% clover	3	7	86	2.2 6	5.7	0.2 7	1.2 5	76	73	14
11	Grasslan d (189/94)	Grass without clover	7	7	83	1.5 7	6.2	0.5 7	1.2 2	35	48	18

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12	Grassland (0/63)	Grass with < 50% clover	7	12	77	2.2 0	7.7	1.1 3	1.4 4	26	44	27
<b>Semi-natural</b>												
13	Heath	Heather	3	9	83	2.0 3	4.6	0.1 9	1.3 3	38	48	4.2
14	Heath	Heather	3	9	83	2.2 6	5.0	0.3 0	1.3 7	41	44	3.1
15	Heath	Heather	2	2	96	0.2 3	5.3	0.0 7	1.5 8	3	6.5	<1. 0
16	Forest	Scots pine	5	12	79	2.0 3	4.3	0.3 0	1.2 3	38	41	7.6
17	Forest	Scots pine	1	7	88	2.1 5	4.7	0.2 1	1.2 9	7.5	9.7	1.1
18	Forest	Heather/ European beech	5	7	83	2.3 8	4.6	0.6 6	1.1 4	24	65	9.4
19	Forest	European beech	13	25	59	2.0 9	4.8	0.6 5	1.1 3	34	45	6.1
20	Forest	European beech	11	25	61	1.6 8	4.5	0.4 0	1.2 5	32	46	5.8

<sup>a</sup>OC, organic carbon; EC, electrical conductivity;  $\rho_b$ , bulk density; Al<sub>ox</sub>, Fe<sub>ox</sub>, P<sub>ox</sub> are oxalate-extractable aluminum, iron and phosphorus, respectively.

**Table 2.** Prediction of decomposition rate ( $k$ ) and stabilization factor ( $S$ ) with multiple linear regression analysis (MLR). The adjusted  $R^2$  of MLR with selected one, two and three sets of variables are presented in the first three rows. The “-” indicates that the parameter is negatively correlated to  $k$  or  $S$ . The “+” indicates a positive correlation. *Basics* include eight basic soil physicochemical properties. *Gas transport* includes relative gas diffusivity ( $D_p/D_0$ ) and air permeability ( $k_a$ ). *Land use* refers to the categories of cropland, 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, $\rho_b$	0.44**	pH, $P_{ox}$ , EC, $\rho_b$	0.69***
Basics, Gas transport	pH, EC, $\rho_b$ , $D_p/D_0$ , $k_a$	0.40*	pH, $P_{ox}$ , $Fe_{ox}$ , $Al_{ox}$ , EC, $\rho_b$ , $D_p/D_0$ , $k_a$	0.83***
Basics, Gas transport, Land use	$P_{ox}$ , EC, $\rho_b$ , Land use	0.63**	$P_{ox}$ , $Fe_{ox}$ , $Al_{ox}$ , EC, $\rho_b$ , $D_p/D_0$ , $k_a$ , Land use	0.86***
Variables from the best-fit set				
Basics	$P_{ox}$	-	$P_{ox}$	+
			$Fe_{ox}$	-
			$Al_{ox}$	-
	EC	-	EC	-
Gas transport	$\rho_b$	-	$\rho_b$	-
			$k_a$	+
			$D_p/D_0$	-
Land use	Grassland	+	Grassland	+
	Forest	-	Forest	-
	Heath	-	Heath	-

$Al_{ox}$ ,  $Fe_{ox}$ ,  $P_{ox}$  are oxalate-extractable aluminum, iron and phosphorus, respectively; EC, electrical conductivity;  $\rho_b$ , bulk density.

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .