

1 **Evaluating the Tea Bag Index approach for different management practices in**  
2 **agroecosystems using long-term field experiments in Austria and Sweden**

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24

25 **Abstract**

26 Litter decomposition is an important factor affecting local and global C cycles. It is known that  
27 decomposition through soil microbial activity in ecosystems is mainly influenced by soil type and  
28 climatic conditions. However, for agroecosystems, there remains a need for a better understanding  
29 how management practices influence litter decomposition. This study examined the effect of  
30 different management practices on decomposition at 29 sites with long-term (mean duration of 38  
31 years) field experiments (LTEs) using the Tea Bag Index (TBI) protocol with standard litter  
32 (Rooibos and Green tea) developed by Keuskamp et al. (2013). The objective was to determine if  
33 the TBI decomposition rate ( $k$ ) and stabilization factor ( $S$ ) are sensitive enough to detect  
34 differences in litter decomposition between management practices, and how they interact with  
35 edaphic factors, crop type and local climatic conditions. Tea bags were buried and collected after  
36 ~90 days in 16 Austrian and 13 Swedish sites. The treatments at Austrian LTEs focused on mineral  
37 and organic ~~fertilization~~fertilizer application, tillage systems and crop residues management,  
38 whereas ~~the Swedish LTEs~~those in Sweden addressed cropping systems, mineral  
39 ~~fertilization~~fertilizer application and tillage systems. The results showed that in Austria,  
40 ~~decomposition differed more between sites than between treatments for the same experiment~~  
41 ~~category.~~Incorporationincorporation of crop residues and high N ~~fertilization~~fertilizer application  
42 increased  $k$ . Minimum tillage had significantly higher  $k$  compared to reduced and conventional  
43 tillage. In Sweden, ~~litter decomposition differed more between treatments than between sites.~~  
44 ~~Fertilized~~fertilized plots showed higher  $S$  than non-fertilized plots and high N ~~fertilization~~fertilizer  
45 had the highest  $k$ . Growing spring cereal lead to higher  $k$  than forage crops. Random Forest

46 regressions for Austria and Sweden jointly showed that  $k$  and  $S$  were mainly governed by climatic  
47 conditions, which explained more than 70% of their variation. However, under similar climatic  
48 conditions, management practices strongly influenced decomposition dynamics. Thus, the TBI  
49 approach may be suitable to apply in a more large-scale network on LTEs for evaluating  
50 decomposition dynamics in agroecosystems more precisely.

51

## 52 **Introduction**

53 Soil organic carbon (SOC) is one of the most used indicators for soil quality, since its dynamics is  
54 involved in regulating ecosystem functionality through its influences on physical, biological and  
55 chemical soil properties, which are critical for nutrient cycling and soil fertility (Davidson et al.  
56 2006; Janzen 2015). Management practices, such as ~~fertilization~~fertilizer application, use of catch-  
57 and cover crops, organic amendments, length of bare fallow periods, permanent surface protection  
58 with perennial crops, tillage practices and aboveground crop residue management, are impacting  
59 SOC balances for agroecosystems (Kätterer and Bolinder, 2022; Sandén et al., 2018; Paustian et  
60 al., 2016).

61 Agricultural soils play a crucial role in the global carbon (C) cycle due to their C sink capacity  
62 (Paustian et al., 2016; Lal, 2004). In this context, improved management practices that maintain  
63 or increase SOC stocks are considered essential in national greenhouse gas reporting systems  
64 (IPCC, 2006), as well as in other international incentives such as the 4 per mille initiative (Minasny  
65 et al., 2017). The SOC balance is dynamic and determined by the difference between annual C  
66 inputs to soil, and the annual C outputs through the decay of existing soil organic matter and ~~newly~~  
67 ~~added litter~~resulting from microbial activity, which is the main contributor to stable SOC  
68 (Tiefenbacher et al., 2021; Bolinder et al., 2007). Management practices have a great impact on

69 these two factors by affecting either the amount of C inputs or outputs through decomposition, or  
70 both factors simultaneously.

71 Litter decomposition is a complex biogeochemical process controlled by several biotic and abiotic  
72 factors, where the biological activity of decomposers varies with soil properties and is driven  
73 largely by climatic conditions (Daebeler et al., 2022; Bradford et al., 2016; Cleveland et al., 2014;  
74 Gholz et al., 2000). Decomposition and SOC stabilization are long processes~~is an extended~~  
75 ~~process~~, therefore long-term field experiments (LTEs) are among the most useful resources for  
76 quantifying the impact of management practices on litter decomposition, SOC changes, and soil  
77 functioning (Sandén et al., 2018; Kätterer et al., 2012; Bergkvist and Öborn, 2011). Experiments  
78 determining litter mass loss over time *in situ* are also important for understanding SOC dynamics,  
79 nutrient cycling and colonization by soil biota under field conditions. The traditional method that  
80 has been used in ecology for more than 50 years consists of litterbag studies, burying known  
81 quantities of various organic materials into the soil, and retrieving them successively at different  
82 intervals (Kampichler and Bruckner, 2009; Burgess et al., 2002; Bockock and Gilbert, 1957). These  
83 studies are not always comparable because they are subject to variations in e.g., litter type, mesh-  
84 size, sample preparation and analytical methods, and the placement of litterbags may alter the  
85 microclimate for decomposers (Kampichler and Bruckner, 2009).

86 Keuskamp et al. (2013) developed therefore a low-cost and time-efficient methodology called Tea  
87 Bag Index (TBI), characterizing the decomposition process with commercially available tea bags,  
88 where green tea is representing labile organic material and rooibos tea as a surrogate for  
89 recalcitrant litter. A decomposition rate ( $k$ ) and a stabilization factor ( $S$ ) are obtained accordingly  
90 with their chemical composition and the respective weight lost at a single point in time after an  
91 incubation period of ca 90-days in the soil. The TBI approach is particularly useful for assessing

92 geographical differences in decomposition dynamics because results are directly comparable  
93 across sites, varying only with local edaphic and seasonal environmental conditions (Keuskamp et  
94 al., 2013).

95 In several studies, the TBI has been used as an indicator for biological (Sandén et al., 2021;  
96 Costantini et al., 2018) and microbial activity (Daebeler et al., 2022; Treharne et al., 2019; Tóth et  
97 al., 2017), ecosystem functioning, nutrient cycling (Zaller et al., 2016), and soil quality (Tresch et  
98 al., 2018; Buchholz et al., 2017). Generally, this follows the concepts of soil quality as reviewed

99 by Bünemann et al. (2018), where TBI would primarily be a biological soil quality indicator. ~~Most,~~  
100 although the data apparently were not normalized for the temperature effect. According to the TBI  
101 community, data collected from networks of researchers and citizen scientists for constructing a  
102 global TBI map (www.teatime4science.org) shows that most studies have been using the TBI  
103 approach for different forest and grassland ecosystems (~~Djukie et al., 2018~~) or urban soils (~~Pino et~~  
104 ~~al., 2021~~). Only, agricultural fields represent less than 15% of data. Indeed, there are only a few  
105 published studies (Daebeler et al., 2022; Dossou-Yovo et al., 2022; Struijk et al., 2022; Fu et al.,  
106 2021; Sandén et al., 2020; Barel et al., 2019; Poeplau et al., 2018; Sievers and Cook, 2018) that  
107 have been using the TBI approach for evaluating agroecosystems, and it is not clear if this method  
108 is sensitive enough to detect differences between management practices.

109 This study used the TBI approach for investigating the effect of management practices on the  
110 decomposition rate ( $k$ ) and stabilization factor ( $S$ ) at several LTEs in Austria and Sweden. ~~,~~ with  
111 different soil characteristics, and climatic conditions, and subjected to various  
112 management/treatments.

113 To the best of our knowledge, this is the first analysis using the TBI approach for such a large  
114 number of LTEs and different treatments. for agroecosystems. The treatments covered

115 management practices such as organic amendments, crop rotations, aboveground crop residue  
116 handling, mineral ~~fertilization~~fertilizer application, and tillage. Our objectives were: (i) to  
117 evaluate: ~~(i)~~ if the TBI  $k$  and  $S$  parameters are sensible enough to ~~detect~~distinguish between  
118 different management practices ~~for agroecosystems~~; (ii) to quantify the effect of management  
119 practices on  $k$  and  $S$ ; and (iii) ~~and~~ to identify the most important local climate and/or soil properties  
120 affecting litter decomposition in Austria and Sweden.

121

## 122 **Materials and Methods**

123

### 124 **Study sites**

#### 125 *Austria*

126 We used sixteen Austrian (AT) sites, by selecting contrasting treatments from three different  
127 categories of LTEs where the management practices had been in place for 11 to 63 years (see  
128 details in Table 1). The TBI measurements were made in 2014, 2015 and 2016. Measurements  
129 sometimes took place in more than one year at the same LTE (e.g., MUBIL), and the sites were  
130 abbreviated as AT1 to AT16. Six experiment categories involved ~~C~~carbon balance practices (CB;  
131 ~~AT1 to AT6~~); focusing on organic matter inputs such as compost and crop residues, eight sites  
132 were studying ~~soil~~soil fertility (SF; ~~AT7 to AT14~~) and in terms of differences in mineral N and  
133 P fertilization, and two sites examined tillage systems (TS; ~~AT15 to AT16~~). The sites are located  
134 in several agricultural areas across the country (Fig. 1), with diverse soil textures (Table S1) and  
135 variable crop types (Table 1) and climatic characteristics (Table 23) during the years of TBI  
136 measurements. More details for some of the sites are available in specific publications: AT3  
137 (Spiegel et al., 2018; Lehtinen et al., 2017; Tatzber et al., 2015; Aichberger and Söllinger, 2009),  
138 AT4 to ~~6~~AT6 (Spiegel et al., 2018; Lehtinen et al., 2014), AT15 and AT16 (Tatzber et al., 2015;

139 Spiegel et al., 2007). In addition, Sandén et al. (2018) puts the Austrian LTEs in the context of  
140 other European LTEs.

141 The purpose of the Austrian C balance LTEs was threefold: i) to assess the sustainability of  
142 stockless vs. livestock-keeping organic farming management on soil and crop traits (AT1, AT2),  
143 ii) to investigate the effects of compost amendments on soil and crops (AT3), and iii) to compare  
144 crop residue incorporation and removal (AT4-AT6). Compost amendment LTE and crop residue  
145 incorporation LTEs also included ~~also~~-mineral ~~fertilization~~fertilizer application, whereas AT1 and  
146 AT2 only focused on different organic ~~fertilization~~fertilizer treatments.

147 The eight soil fertility LTEs (AT7-AT14) were all focusing on the effect of mineral ~~fertilization~~  
148 fertilizer on soil and crop properties. In most cases, treatments studied different amounts of mineral  
149 nitrogen ~~fertilization~~fertilizer, whereas AT9 and AT12 also investigated the effect of different  
150 amounts of K ~~fertilization~~fertilizer application. Nitrogen ~~fertilization~~fertilizer was applied in four  
151 stages and potassium in three stages, according to Austrian guidelines for ~~fertilization~~fertilizer  
152 (BMLFUW, 2017).

153 For the tillage system experiments, conventional tillage (CT) was compared to reduced tillage (RT)  
154 and minimum tillage (MT). Regular mouldboard ploughing to 25–30 cm soil depth was applied in  
155 CT treatment, whereas cultivator in autumn to a depth of 15–20 cm was used in RT treatment and  
156 a rotary driller that loosened the soil to a depth of 5–8 cm was used in MT treatment. The soil was  
157 turned over only in the CT treatment, where inversion tillage was incorporating the crop residues.  
158 ~~Fertilization~~Fertilizer application was crop specific according to the Austrian guidelines for  
159 ~~fertilization~~fertilizing (BMLFUW, 2017).

160

161 *Sweden*

162 We used thirteen Swedish (SE) sites by selecting contrasting treatments from three different  
163 categories of LTEs, where the management practices had been in place for 11 to 59 years (see  
164 details in Table 2). TBI measurements at these sites were made only in one year (2016), and were  
165 abbreviated as SE1 to SE13. Six sites involving combined management practices (CMP, ~~SE1 to~~  
166 ~~SE6~~), four studying the effect of rotations (ROT, ~~SE7 to SE10~~), and three sites with tillage systems  
167 (TS, ~~SE11 to SE13~~). The sites are located in several agricultural areas across the country (Fig. 1),  
168 with diverse soil textures and variable crop types (Table 2) and climatic characteristics (Table 3)  
169 during the year of TBI measurements. Bergkvist and Öborn (2011) give a general description of  
170 all these LTEs. Only a brief description of treatments are provided below, more details on the  
171 sites with combined management practices is given by Carlgren and Mattson (2001), and for tillage  
172 systems by Arvidsson et al. (2014), while Poeplau et al. (2015) provide some more insight on the  
173 rotation experiments.

174 The initial purpose of the six LTEs with combined management practices (SE1-SE6) was to  
175 compare a change from the traditional mixed farm production system including crops and livestock  
176 into a pure cash crop system, by studying their effects on the sustainability of crop production and  
177 soil properties (entitled *soil fertility experiments*). The dairy production treatments contain  
178 exclusively perennial grass-clover leys and ~~receives~~receive one farmyard manure (FYM)  
179 application per rotation. The cash crop treatments consist of annual crops (i.e., oilseed is replacing  
180 leys in the rotation) without manure applications (0 FYM) only receiving mineral fertilizers  
181 application (NPK). PK applications in all the treatments we selected were aimed at achieving rapid  
182 build-up of the soil PK status, i.e., the amount applied was first replacing that exported in harvested  
183 products (i.e., maintenance principle), to which an extra amount was added (corresponding to the  
184 max treatment). The N-rates in all NPK treatments were also corresponding to max application



185 rate, and were adapted depending on crop type, where spring cereals, oilseeds, and leys received  
186 125 kg, while sugar beet received 210 kg N ha<sup>-1</sup> yr<sup>-1</sup>. We were also using the control plots receiving  
187 no NPK (0 NPK). As a third factor ~~in these CMP~~, aboveground crop residue removal takes place  
188 in all FYM treatments, simulating use of harvest residues for fodder or bedding material that are  
189 recycled as manure. The southern sites have 4-year rotations and those in central Sweden have 6-  
190 year rotations. The north site (SE5) is slightly different from the others, consisting of a 7-year  
191 rotation and is studying only the livestock-based production system.

192 ~~We~~For rotation experiments purposes, we were comparing extreme treatments representing two  
193 rotations from ~~three~~four LTEs (SE7-SE10) with the main objective to study changes in SOC  
194 (named *humus balance experiments*), i.e., a continuous spring cereal (SC) system and a ley-  
195 dominated rotations (L). The straw was removed from the plots every year in the SC treatments,  
196 and L consisted of a grass-clover mixture re-established every fourth year. Both rotations were  
197 receiving P and K accordingly with the maintenance principle, and SC and L were receiving 120  
198 and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively.

199 In the ~~three~~ tillage experiments, (SE11-SE13), the conventional tillage (CT) and direct seeding  
200 (DS) treatments were the same for all sites, consisting of inversion ~~ploughing~~plowing to a depth  
201 of 20-23 cm and by using a disc seed drill, respectively. The shallow (5-7 cm) and deep (~12 cm)  
202 reduced tillage treatments (SRT and DRT, respectively), consisted of primary tillage operations  
203 made in the autumn and most commonly with a chisel plough. The main crops in all the tillage  
204 system experiments were winter and spring cereals (occasionally oilseed), fertilized accordingly  
205 with local recommendations and with the aboveground residues chopped and left in the field.

206

207 **TBI method and sampling design**

208 The TBI method was used according to the protocol established by Keuskamp et al. (2013) to  
209 determine litter decomposition using two types of commercial tetrahedron-shaped tea bags by  
210 Lipton Unilever (Green tea and Rooibos tea). The green tea (*Camellia ~~sinensis~~sinensis*; EAN:  
211 8722700055525) has high cellulose content, higher soluble fraction, and lower C:N ratio; while  
212 rooibos tea (*AspalanthusAspalathus linearis*; EAN: 8722700188438) has high lignin content,  
213 lower soluble fraction, and higher C:N ratio, which is expected to slow down decomposition  
214 (Keuskamp et al., 2013). The synthetic tea bag material has a mesh size opening of 0.25 mm  
215 allowing access to microorganisms, very fine roots and root hairs.

216 The initial mass of the tea bag contents was determined on 20 randomly selected bags for each tea  
217 type from different boxes, oven-dried at 70°C for 48 hours and weighed separately; the mean dry  
218 mass for green tea was  $1.717 \pm 0.048$  g and that for rooibos tea was  $1.835 \pm 0.027$  g. For both  
219 countries, close to seeding of annual crops, from end of April to mid-June depending on location,  
220 each tea bag was properly identified and buried in the soil at 8 cm depth.

221 For Austria, ~~only one site used successive retrieval dates (AT16), in which~~ four bags of each tea  
222 were used and placed side-by-side at a distance of 2 to 3 cm, ~~in order to keep as similar soil~~  
223 ~~characteristics as possible. In this case, the~~ Each tea bags collecting occurred after 16, 26, 62 bag  
224 was properly identified and buried in the soil at 8 cm depth. The91 days. ~~For the other Austrian~~  
225 ~~sites, there was only one collecting, and the~~ TBI incubation period from placement to last retrieval  
226 averaged  $80 \pm 13$  days (Table 3) ~~due to logistic issues for collection~~. After collecting, the tea bags  
227 were ~~cleaned of soil and roots and~~ oven-dried at 70°C for 48 hours after removal of adhered soil  
228 particles according to the standardized protocol by Keuskamp et al. (2013). After drying, the tea  
229 bags were opened and the tea content was weighted. The ash content was not determined.

230 The same TBI protocol was used for the Swedish sites ~~but all the sites used successive retrieval~~  
231 ~~dates.~~ As in Austria, four bags of each tea were used per experimental unit ~~for each retrieval date,~~  
232 placed side-by-side at a distance of 2 to 3 cm. Each tea bag was properly identified and buried in  
233 the soil at 8 cm depth. ~~The tea bags were collected after four different time periods of ~15, 30, 60~~  
234 ~~and 90 days.~~ The mean TBI incubation period from placement to last retrieval date averaged  $91 \pm 1$   
235 day (Table 3). ~~To quantify~~ In addition to the removal of adhering soil contamination particles, the  
236 ash content was determined ~~for each of the four retrieval dates~~ (i.e., both for green and rooibos tea  
237 on mixed samples of the four replicates) in a muffle oven at 550°C for 16 hours. The rationale for  
238 measuring the ash content was that three of the Swedish sites had a high clay content (Table S1),  
239 where the complete removal of adhering soil particles may be more difficult. However, the ash  
240 content was on average quite low, representing  $15 \pm 6\%$  and  $10 \pm 4\%$  for the Green and Rooibos tea  
241 bags, respectively (data not shown).  
242 ~~After measuring the remaining dry matter, the decomposition rate ( $k$ ) and stabilization factor ( $S$ )~~  
243 ~~were calculated according to Keuskamp et al. (2013).~~  
244 After measuring the remaining dry matter, the decomposition rate ( $k$ ) and stabilization factor ( $S$ )  
245 for both countries were calculated according to the TBI presented by Keuskamp et al. (2013). This  
246 standardized method that is using single measurements after an incubation period in the soil of 90-  
247 days have received some criticism. For instance, Mori (2022) and Mori et al. (2023) showed that  
248 this incubation period is not always long enough for the mass loss of green tea to reach a plateau,  
249 and further suggested that time-series mass loss data of rooibos tea is also required to respect the  
250 underlying assumptions of the TBI method. Time-series (15, 30, 60 and 90-days) of green and  
251 rooibos tea were available for all the Swedish sites but only at one Austrian site (16, 26, 62 and  
252 91-days at AT16). The incubation period was consistently always 90-days for the Swedish sites,

253 and only shorter than that (i.e., about 60-days incubation period) for a few of the Austrian sites  
254 (Table 3). To have as uniform comparisons as possible between the two datasets, we only used the  
255 last measurement for both countries for calculating  $k$  and  $S$ . The purpose of using the time-series  
256 for testing the underlying TBI assumptions was beyond the scope of this paper.

257 The daily climate data for Austria were retrieved from the Central Institution for Meteorology and  
258 Geodynamics (ZAMG). For Sweden, the daily climate data were gathered through official data  
259 from the most nearby LantMet climate stations, and from the Swedish Meteorological and  
260 Hydrological Institute (SMHI). The climate variables used in this study were air temperature,  
261 precipitation, solar radiation, wind speed and air humidity (Table 3).

262 For Austrian soils, pH was measured electrochemically (pH/mV Pocket Meter pH 340i, WTW,  
263 Weilheim, Germany) in 0.01 M CaCl<sub>2</sub> at a soil-to-solution ratio of 1:5 (ÖNORM L1083). Total  
264 soil organic C (TOC) concentrations were analyzed by dry combustion in a LECO RC-612 TruMac  
265 CN (LECO Corp., St. Joseph, MI, United States) at 650°C (ÖNORM L1080). Total N (N<sub>tot</sub>) was  
266 determined according to ÖNORM L1095 with elemental analysis using a CNS (carbon, nitrogen,  
267 sulfur) 2000 SGA-410-06 at 1250°C. Texture was determined according to ÖNORM L1061-1 and  
268 L1062-2. For Sweden the data were gathered from recent archived analysis protocols: (pH was  
269 measured in water). Clay content, C content, C:N ratio, and pH measured from each site in both  
270 countries are shown in Table S1 (Supplementary material).

271

## 272 **Data analysis**

273 Analysis of variance (ANOVA) for each experiment category (i.e., CB, SF, TS, CMP and ROT)  
274 was performed to analyze ~~treatment and site~~ the effects of the treatments and the differences  
275 between sites on  $k$  and  $S$  separately for both countries. When the treatments were identical within  
276 the same experiment category, sites were used as a random effect with a mixed ANOVA to test

277 the average treatment effect, mean values were used as replicates to test the differences between  
278 sites. The ~~-, followed by the~~ Tukey's test ( $p < 0.05$ ) was used for comparing the same treatments  
279 and the same sites using R software version 4.2.2. We have treated the data from different years at  
280 the same sites in Austria as independent observations, in the sense they are not a time series of  
281 measurement. Interactions between site and treatment were considered.

282 We ~~used~~calculated a climate-dependent soil biological activity ~~scaling function~~parameter ( $Re_{clim}$ ),  
283 ~~which is included in the ICBM SOC model (Andrén and Kätterer, 1997) for adjusting the~~  
284 ~~decomposition rates of SOC pools (Andrén et al. 2004; 2007). This function~~by using mean daily  
285 air temperature, total precipitation, and potential evapotranspiration (PET) data in pedotransfer,  
286 soil water balance and biological activity functions. Compared to raw climatic data alone, this  
287 parameter is integrating the effect of climate, soil and crop properties ~~by calculating.~~ It is calculated  
288 as the product of a soil temperature ( $Re_{temp}$ ) and relative water content ( $Re_{wat}$ ) ~~in the arable layer.~~  
289 factor with a daily time step (i.e.,  $Re_{clim} = Re_{temp} \times Re_{wat}$ ), which is thereafter averaged to give an  
290 estimate of soil biological activity for a given time period. These two ~~variables~~factors are derived  
291 from soil temperature and soil moisture response functions expressing the activity of decomposers  
292 and their relative effect on ~~decomposition kinetics. The~~the decay rates of organic materials in the  
293 arable layer of agricultural soils. Briefly, the  $Re_{temp}$  is calculated from air temperature and leaf area  
294 index using an empirical model (Kätterer and Andrén, 2009), while  $Re_{wat}$  is calculated using  
295 pedotransfer functions for simulating the soil water balance and a function for estimating ~~potential~~  
296 ~~evapotranspiration (PET).~~PET. In addition to air temperature and leaf area index, calculations of  
297  $Re_{wat}$  also involve the use of daily climatic data for precipitation, wind speed, air humidity, and  
298 solar radiation, as well crop types and yields, soil texture and SOC content ~~(for details see Bolinder~~  
299 ~~et al., 2008, and Fortin et al., 2011).~~ For details see Bolinder et al., 2008, and Fortin et al., 2011)

300 and information in the following R package used for the calculations:  
301 <https://github.com/ilmenichetti/reclim> (Please refer to the package documentation). The  $Re_{clim}$   
302 concept can be used for quantifying regional differences in soil biological activity alone (Bolinder  
303 et al., 2013; Andrén et al. 2007) or integrated as a parameter in the ICBM SOC model. In the latter  
304 case, it is adjusting the decomposition rates of both C inputs to soil from crop residues (e.g., straw)  
305 and that of the more stable SOC (Andrén and Kätterer, 1997; Andrén et al., 2004). In this study,  
306 we used the concept of  $Re_{clim}$  and the  $Re_{temp}$  and  $Re_{wat}$  factors to test if the product of soil-  
307 temperature and relative water content better explained the variation in  $k$  and  $S$  than the two latter  
308 alone, and to determine if they also better explained this variation compared to using only raw  
309 climatic data.

310 ~~We calculated simple correlation between variables using Pearson correlation.~~We calculated  
311 simple correlation between variables mean annual temperature (MAT), mean temperature during  
312 the incubation period ( $MAT_{TBI}$ ), total annual precipitation (TAP), total precipitation during  
313 incubation period ( $TP_{TBI}$ ), potential evapotranspiration (PET), potential evapotranspiration during  
314 incubation period ( $PET_{TBI}$ ), aridity index (AI), aridity index during incubation period ( $AI_{TBI}$ ),  
315 temperature and precipitation factor (TxP),  $Re_{clim}$ ,  $Re_{wat}$ ,  $Re_{temp}$ , pH, SOC, clay and C:N ratio,  
316 using Pearson correlation. For more accurate results, we applied random forest (RF) regression in  
317 order to rank the importance of variables for  $k$  and  $S$ , using the random forest R package (Liaw  
318 and Wiener, 2002). The RF is a machine learning technique based on decision trees that predicts  
319 a certain variable from a set of other variables through a series of binary splits of the data, where  
320 the variables are either continuous or categorical. For example, in the case of a continuous variable  
321 it consists of all the data points above or below a certain threshold, for a categorical variable it  
322 consists of all the data points belonging or not to a specific class. All these subsequent splits

323 constitute a decision tree. A random forest is a set of decision trees and it is therefore an ensemble  
 324 technique. This allowed us ~~utilizing to utilize~~ treatment and crop variables (including N  
 325 ~~fertilization fertilizer~~) without having to convert them into a ranking. Another useful asset of an RF  
 326 regression is that it evaluates the importance of each variable in defining the predicted variable.  
 327 There are various possible measurements to do that, but they are all based on measuring the  
 328 effectiveness of each subsequent split in each node of a decision tree in sorting out the information.  
 329 In our study, we used a measurement called node purity based on the Gini index, which expresses  
 330 the probability of one split of the data (i.e., one node of the tree) defining the predicted variable.  
 331 The total node purity of a certain variable in a tree is the sum of all the node purity measurements  
 332 for each node considering that particular variable, and the higher it is the more that variable is  
 333 important.

334 We used the following models to predict the two TBI ~~kinetic parameters  $k$  and  $S$  (considering data~~  
 335 ~~from measurements only at 60 days and only at 90 days, or all measurements from 60 and 90 days~~  
 336 ~~combined): parameters  $k$  and  $S$ :~~

337  
 338  ~~$k_n f(TN, N, SOC, PET_{TBI}, PET, cr, TP_{TBI}, TAP, tr, cl, CN, pH, MAT, MAT_{TBI}, AI, AI_{TBI}, Re_{clim}, Re_{wat}, Re_{temp})$~~

339  ~~$S_n f(TN, N, SOC, PET_{TBI}, PET, cr, TP_{TBI}, TAP, tr, cl, CN, pH, MAT, MAT_{TBI}, AI, AI_{TBI}, Re_{clim}, Re_{wat}, Re_{temp})$~~

340  
 341 ~~where the subscript  $n$  denotes the grouping based on how long period the tea bags were in the soil~~  
 342 ~~(i.e., for 60 or 90 days, or both periods combined). Soil~~

343  ~~$k_n f(TN, SOC, CN, cl, cr, tr, pH, N, PET, PET_{TBI}, TAP, TP_{TBI}, MAT, MAT_{TBI}, AI, AI_{TBI}, Re_{clim}, Re_{wat}, Re_{temp})$~~

344  ~~$S_n f(TN, SOC, CN, cl, cr, tr, pH, N, PET, PET_{TBI}, TAP, TP_{TBI}, MAT, MAT_{TBI}, AI, AI_{TBI}, Re_{clim}, Re_{wat}, Re_{temp})$~~

345

346 where soil variables (continuous) were  $TN$  (total N g kg<sup>-1</sup>),  $SOC$  (g kg<sup>-1</sup>),  $CN$  (C:N ratio),  $cl$  (clay  
347 content g 100 g<sup>-1</sup>) and  $pH$ . Categorical variables were  $N$  (N ~~fertilization~~fertilizer application factor  
348 with 4 levels),  $cr$  (~~a~~-crop factor, e.g., barley, ley (establishment), ley (production), oat, spring  
349 oilseeds, sugar beet, maize and winter wheat) and  $tr$  (a treatment factor with 30 levels). The  
350 climatic variables ( $PET_{TBI}$ ,  $PET$ ,  $TP_{TBI}$ ,  $TAP$ ,  $MAT_{TBI}$ ,  $MAT$ ,  $AI_{TBI}$  and  $AI$ ) are as defined in Table  
351 3. The climate response variables  $Re_{clim}$ ,  $Re_{wat}$ ,  $Re_{temp}$  are as described above.

352 Since many of variables in our model are likely to be correlated and carry similar information, we  
353 applied the recursive feature elimination algorithm implemented in the caret R package by Kuhn  
354 et al. (2016), which assess in subsequent iterations the optimal set of predicting variables (features)  
355 to be utilized by the RF model. The procedure starts by fitting a RF model with all variables,  
356 ranking them by importance, and discarding the least important. The algorithm then iterates. The  
357 optimal number and set of features are then defined by a fitness metric (in our case the model  $R^2$ ),  
358 selecting the set with the best model fitness. The selected models were used to compute the  
359 variables' relative importance.

360 In order to better understand the similarities between the sites, we run a principal component  
361 analysis (PCA) in the space composed by the variables pH, SOC, TN, C:N, clay, TAP, MAT, PET,  
362 AI and TxP. The analysis was run with the R command prcomp (Venables and Ripley, 2002), from  
363 the base R installation (“Stats” package). Variables were all standardized by rescaling them to zero  
364 mean and unit variance.

365



## 366 Results

### 367 *Effect of management practices*

#### 368 *Austria*

369 Both the TBI parameters  $k$  and  $S$  varied between treatments and sites in Austria, and even between  
370 years at the same site within the C balance category (Fig. 2 and Table ~~S3S2~~). In general, all  
371 treatments in AT1 with a lucerne crop under wetter conditions (2014) presented higher  $k$  and lower  
372  $S$  than in AT2 with a wheat crop under dryer conditions (2015), and the ~~FW~~-treatment with  
373 municipal compost and green manure (FW) had the highest  $S$  in 2015. The AT3 site did not present  
374 significant differences between the treatments. ~~Treatment-Crop residue incorporation treatment~~  
375 (CRI) had higher  $k$  than the crop residue removal (CRR-treatments) at the AT4 and AT6 sites, and  
376 AT6 presented a higher  $k$  than at AT4. Comparing years for the same experiment site and type  
377 with different crops, AT5 (2015; with wheat) had higher  $S$  than AT6 (2016; with maize).

378 For the soil fertility experiment category (Table 1), AT12 had the highest  $k$  and AT13 had the  
379 highest  $S$ . Sites receiving NPK ~~fertilization~~fertilizer application (AT7, AT8 and AT9) had higher  
380  $k$  and  $S$  even at different N doses than at the AT12, AT13 and AT14 sites receiving only N (i.e.,  
381 without P and K). Stabilization was significantly higher in AT9 than in AT7. For ~~K~~potassium  
382 trials, AT11 presented significantly higher  $k$  and  $S$  than AT10. Regarding sites receiving only N  
383 addition-~~only~~ (AT12, AT13, and AT14), maximum doses (180 kg N) presented the highest  $k$   
384 (0.0095), and no N addition had the lowest  $k$  (0.0066) (Fig. 2 and Table S2).

385 Regarding the tillage system experiment category ~~at the Fuchsenbigl LTE~~ (AT15 and AT16 -  
386 Table 1),  $k$  was significantly higher in shallow reduced tillage (SRT) and  $S$  was higher in deep  
387 reduced tillage (DRT) in 2015 (AT15), but no significant differences between treatments were  
388 found in 2016 (AT16). Site AT16 had significantly higher  $S$  than AT15 (Fig. 2. and Table S2).

389

390 *Sweden*

391 At the Swedish sites (Table 2), the ~~90-days~~TBI measurements for the combined management  
392 practices experiment category showed that both  $k$  and  $S$  were significantly higher for the  
393 FYM/NPK treatments maximum amount of farmyard manure and maximum doses of NPK  
394 (FYM/NPK) (Fig. 3, Table S3) compared with the control treatments (0 FYM/0 NPK).

395 Comparing sites, SE5 and SE6 had the highest  $k$  and SE4 had the lowest  $k$ , while SE3 presented  
396 the highest  $S$  followed by SE6, SE5 and SE4,  ~~$S$  was~~the lowest  $S$  was for SE1 and SE2 (Table S3).

397 Regarding the rotation experiments, the continuous spring cereal rotation presented higher  $k$  than  
398 for ley, but there was no significant difference in  $S$ . Comparing sites, SE9 presented higher  $k$  than  
399 SE7, SE8 and SE10, whereas SE7 and SE8 had the highest  $S$ .

400 For the tillage system experiment category, conventional tillage (CT) had the lowest  $k$  and  $S$ , while  
401 deep reduced tillage (DRT) had the highest  $k$  and  $S$ . The highest  $S$  was observed for the SE12  
402 followed by SE11 and SE13. Sites did not show significant differences for  $k$ .

403 Comparing tillage system experiments in Austria and Sweden (2016) for sites (AT16, SE11, SE12  
404 and SE13) ~~and~~for the treatments that were the same in both countries (CT, SRT and DRT),  
405 ~~DRT~~deep reduced tillage had ~~higher~~the highest  $k$  and ~~SRT~~shallow reduced tillage had ~~higher~~the  
406 highest  $S$ . The Austrian site presented the lowest  $k$  and the highest  $S$  compared to the Swedish  
407 sites, which did not present significant differences among them (Table S3).

408 ~~Mean~~The field application of the TBI found a clear discrimination of both  $k$  and  $S$  values between  
409 the two countries. The both mean  $k$  and  $S$  values were higher in Sweden (Table 4, Fig. 4). In  
410 general, the variation in  $k$  and  $S$  values were lower in Austria. Indeed, mean  $k$  by site in Austria  
411 varied between 0.0053 and 0.0058 and 0.0149, 0.0128 and mean  $S$  varied between 0.113 and 0.442

412 (Table S3). ~~Mean S2). Whereas mean  $k$  by site in Sweden was between 0.0084 and 0.03110301,~~  
413 and mean  $S$  was between 0.125118 and 0.365361 (Table S3). All values for  $k$  and  $S$  were within  
414 the range of the previous global TBI investigation (0.005-0.04 for  $k$ ; and 0.05-0.55 for  $S$ ) by Sandén  
415 et al. (2020).

416 ~~The mean values of the TBI decomposition rate and the stabilization factor were both higher at 60~~  
417 ~~days than at 90 days, in Austria as well as in Sweden (Table 4). After 90 days of incubation, mean~~  
418  ~~$k$  was higher in Sweden and mean  $S$  was higher in Austria. Applying a decomposition model to~~  
419 ~~the series of data from successive retrieval dates (i.e., all the Swedish sites and the AT16 site in~~  
420 ~~Austria) on the remaining dry matter over time showed faster decomposition of Green compared~~  
421 ~~to Rooibos tea, which is in agreement with the TBI concept (Fig. S1). Whereas the decomposition~~  
422 ~~curve for Rooibos kept decreasing after 90 days, that of green tea did not decrease any further after~~  
423 ~~about 60 days. The variability between sites in dry matter loss over time was higher for Green than~~  
424 ~~for Rooibos tea. The field application of the TBI found a clear discrimination of both  $k$  and  $S$~~   
425 ~~between agroecosystems in Austria and Sweden after the incubation period (Fig. 4).~~

426

#### 427 *Influence of climate and soil properties*

428 Using the combined dataset for ~~the 90 days TBI period~~ Austria and Sweden, resulted in significant  
429 negative correlation between  $k$  and MAT, TAP, PET, TxP factor,  $Re_{clim}$  and  $Re_{temp}$ , and significant  
430 positive correlation with the C:N ratio (Table S4). Stabilization factors in Austria and Sweden  
431 combined correlated negatively with MAT<sub>TBI</sub> period,  $Re_{clim}$  and  $Re_{temp}$ . ~~After the 60 days TBI~~  
432 ~~period, Austria and Sweden combined presented significant negative correlation between  $k$  and~~  
433 ~~MAT, PET, AI, T x P factor, pH and clay content, and a positive correlation with TAP and C:N~~  
434 ~~ratio. Stabilization correlated negatively with the C:N ratio.~~

435 The variable selection procedure with the random forest models (Fig. 5) identified fewer variables  
436 explaining  $k$  and  $S$  values for the combined dataset. ~~When considering only the 90 days subset,~~  
437 ~~the~~The variables explaining  $k$  ~~increased~~is low, but the overall predicting power of the model  
438 decreased substantially. ~~A similar pattern, but less strong, was noticed for the 60 days subset.~~  
439 ~~More than 70%~~ According to the optimized random forest model, most of the variance ~~of  $k$  for the~~  
440 ~~combined dataset (i.e., 60 and 90 days TBI period)~~in  $k$  was accounted for by climatic variables  
441 ~~only~~ (Fig. 65), with  ~~$Re_{wet}$  and  $Re_{temp}$~~  ranking the highest, followed by  ~~$Re_{elim}$~~ , AI and MAT,  
442 ~~according to the optimized random forest model. On the contrary,~~ It was also showing that  
443 treatment, C:N ratio and SOC were important factors as well. The  $S$  was also influenced by ~~much~~  
444 ~~more factors, again with~~ climate-related variables, with  $Re_{temp}$  leading the ranking ~~but including~~  
445 ~~also many edaphic characteristics, such as,~~ and it was followed by pH, SOC, clay and nitrogen  
446 ~~content and the C:N ratio, as well as~~ more agronomic variables such as treatment, crop and N  
447 fertilizer application.  
448 The principal component analysis (Fig. 6) revealed that the data are well divided in two groups,  
449 representing the two countries, which allow us to say that indeed edapho-climatic characteristics  
450 are different between Austria and Sweden. PC1 showed that points in Austria are positively related  
451 to climatic characteristics, such as MAT, TAP and PET. On the other hand, points in Sweden are  
452 positively influenced by high clay content and high C:N ratio. High C:N enhancement is related  
453 to low TAP and TxP. High clay contents are related to low MAT and PET, which can be due to an  
454 historical influence on soil weathering. PC2 is dominated by SOC and TN contents, mainly at two  
455 points in Austria (AT7 and AT9: two sites testing soil fertility with NPK and relatively low TAP  
456 and TP<sub>TBI</sub> compared to other sites). Austria presented more divergent data, having more  
457 heterogeneous sites than in Sweden.

458

459

## Discussion

### Influence of climate and soil properties

In previous studies using the TBI approach, it was shown that climate played a significant role on decomposition in a temperate biome (Djukic et al., 2018), but when comparing several different biomes, climatic conditions were of relatively low importance (Fanin et al., 2020). In Boreal soils, Althuizen et al. (2018) found that increased temperatures enhanced  $k$ , whereas increased precipitation decreased  $k$  across years. Many studies have showed a positive correlation between precipitation and decomposition rates (Pimentel et al., 2019; García Palacios et al., 2016), however precipitation did not show correlation with  $k$  according to the random forest analysis. On the other hand,  $Re_{wat}$  showed great importance (Fig. 5). It is because this variable includes nonlinearities due to its shape according to which decomposition increases with soil moisture and then decreases at high soil water content due to oxygen limitation of microorganisms (Moyano et al., 2013).

~~fertilization. The rankings when using the two subsets of data separately (i.e., 60, and 90 days TBI periods) were less relevant since the predictive power of the In general, higher  $k$  values were observed when the aridity index was lower, and it was identified by the random forest regression model decreased compared to when using the combined dataset, being an important variable affecting the rate of decomposition (Fig. 5). This may be related to the observations that in more arid and warmer sites, the biological processes driving SOC dynamics are impaired which may result in decreasing soil C and N stocks (Kerr and Ochsner, 2020; Ontl and Schulte, 2012; Jiao et al., 2016; Reynolds et al., 2007), was particularly true for  $k$ , where the overall cumulated node purity also decreased substantially compared with the combined dataset.~~

For practical reasons the teabags in our study were buried in the soil during the growing season, corresponding to a period when the soil biological activity is highest (Bolinder et al., 2013). When

483 burying the teabags during the growing season, the difference in climatic variables between sites  
484 are attenuated, in particular with respect to air temperature. For example, the MAT at the Swedish  
485 most northerly (SE5 and SE9) and southerly (SE2) sites are 4.1 and 9.0 °C, respectively, whereas  
486 the corresponding mean air temperatures during the incubation period ( $MAT_{TBI}$ ) were 14.3 and  
487 16.2 °C, respectively.

488 The TBI  $S$  parameter was also dependent on climate. Indeed, the random forest model identified  
489 climatic parameters as the main factors affecting  $S$  in both Austria and Sweden during all evaluated  
490 periods. In particular,  $Re_{clim}$  and  $Re_{temp}$  often showed significant negative correlations, which  
491 implies a negative impact of air temperature on  $S$ . However, raw climatic variables, such as  
492 precipitation and temperature were only weakly correlated with  $S$ . This is probably due to  
493 nonlinear processes, which are considered in the ICBM climate-dependent soil biological activity  
494 calculations such as  $Re_{wat}$  (as discussed above). Furthermore, since litter decomposition dynamics  
495 is influenced by multiple factors that interact and change over time (Bradford et al., 2016), the  
496 relationships are not always linear. Random forest models that we fitted to the data are more  
497 efficient capturing such combinations and interactions of factors, and can detect relationships that  
498 would not be detectable by linear approaches.

499

## 500 **Discussion**

### 501 *Effect of management practices*

502 Our results revealed that a large number of different management practices significantly affected  
503 both the decomposition rate  $k$  and stabilization factor  $S$  according to the TBI approach used in  
504 several LTEs in Austria and Sweden (Fig. 2, 3 and 65). This is in ~~contrary~~contrast to the studies  
505 by Djukic et al. (2018) as well as Saint-Laurent and Arsenault-Boucher (2020), who did not find  
506 any significant effect of land use and management practices on early-stage litter decomposition in  
507 a temperate biome.

508 In the C balance trials in Austria, soils receiving green manure + municipal compost (~~FW~~) had  
509 higher  $S$  than soil receiving biogas slurry (~~BS~~) at the second year (Table S2). This is in agreement  
510 with studies indicating that compost can improve SOC stabilization over time (Mekki et al., 2019;  
511 Eshetu et al., 2013; Ceccanti et al., 2007). The higher  $k$  in the ~~CR~~crop residues incorporated  
512 treatments (AT4 and AT6; Table S2) can be attributed to the fact that incorporation of crop residues  
513 into the soil can increase the decomposition rate by stimulating microbial activity. During the early  
514 stages of decomposition, soluble C is rapidly utilized by ~~soil biota~~soil biota (~~Werth and Kuzyakov,~~  
515 ~~2010~~Werth and Kuzyakov, 2010). The higher  $k$  and lower  $S$  at AT6 compared to AT4 were likely  
516 due to the loamy texture, lower ~~PET~~potential evapotranspiration resulting in ~~a lower~~ Aridity  
517 index at the AT4 site (Table 3 and Table S1), corroborated by the PCA analysis (Fig. 6).

518 There were no significant differences in  $k$  and  $S$  found among treatments in the soil fertility trials  
519 in Austria with NPK addition. However, there was a trend towards a higher  $S$  at AT9 compared to  
520 AT7, likely related to the higher SOC content in AT9, since the climatic conditions and soil texture  
521 were quite similar for both areas, which suggests that higher SOC content may have increased  $S$ .  
522 Site AT11 had higher  $k$  and  $S$  than AT10. Possible explanations for this trend are that AT10 had



523 lower clay content, lower precipitation resulting in higher PET (corroborated by the PCA analysis)  
524 and AI, contributing to a lower soil moisture content and thereby lower decomposition and  
525 stabilization.

526 Nitrogen supply may favor microbial activity and thereby litter decomposition (Raiesi, 2004). This  
527 was reflected in the treatments where only N was added, where the high dose of 180 kg N ha<sup>-1</sup>  
528 (AT12, AT13 and AT14) induced a significantly higher  $k$  (Table S2), compared to the treatments  
529 with no N addition, which also had the lowest  $k$ . Furthermore, the significant difference between  
530 sites, in which AT12 had the highest  $k$  could at least partly be explained by a higher SOC and  
531 higher pH at this site (corroborated by the PCA analysis).

532 In the tillage system experiment at Fuchsenbigl in Austria in 2015 (AT15, with maize) and 2016  
533 (AT16; with wheat), the shallow reduced tillage (~~SRT~~) showed significantly higher  $k$  than ~~DRT~~  
534 deep reduced tillage and ~~CT, but~~ conventional tillage, only in 2015, indicating that shallow soil  
535 tillage stimulated decomposition that particular year. This was likely due to climatic conditions,  
536 since 2015 was slightly drier and warmer, furthermore maize straw has lower C:N ratio than wheat  
537 straw and tends to decompose faster. Some studies showed faster decomposition under  
538 conventional tillage than under reduced tillage practices (e.g., Lupwayi et al., 2004). However,  
539 Kainiemi et al. (2015) found a decrease in soil respiration in conventional tillage compared to  
540 shallow tillage in temperate regions, which directly implies a lower decomposition (and lower  $k$ ).  
541 These differences between tillage treatments are attributable to indirect effects on soil moisture  
542 and temperature profiles. We attribute the significantly higher  $S$  in 2016 to the fact that this year  
543 was moister and less warm, compared to 2015, resulting in a lower ~~AI<sub>TBI</sub>~~ aridity index during the  
544 TBI incubation period: (AI<sub>TBI</sub>).

545 In the Swedish combined management practices trials, ~~soil~~-treatments receiving organic and  
546 mineral ~~fertilization~~fertilizer application had higher  $k$  and  $S$  (FYM/NPK; Table S3), likely due to  
547 the increase in microbial diversity and activity favored by nutrient and C supply (Stark et al., 2007).  
548 Sites SE5 and SE6 presented the highest  $k$ : SE5 had low ~~PET~~evapotranspiration and ~~A~~Acidity  
549 index, resulting in more moisture; SE6 had also high  $S$ , due to high clay content and low PET-  
550 (corroborated by the PCA analysis). Site SE3 had high  $S$ , which could be related to a higher C:N  
551 ratio, as suggested in another TBI experiment by Althuizen et al. (2018), a study showing that  
552 C:N ratio is positively correlated to  $S$ . The SE1 and SE2 sites had lower  $S$  than SE3 despite similar  
553 climatic conditions, which probably was related to the type of crops growing in these treatments  
554 (i.e., sugar beet in the SE1 and SE2 and grass/clover ley in SE3), ~~which~~since these two crop types  
555 have different effects on soil temperature and moisture.

556 In the Swedish rotation system trials, spring cereal (SC) had higher  $k$  than ley (Table S3). Site SE9  
557 had higher  $k$  and lower  $S$ , in which the low stabilization may be caused by low clay content, low  
558 pH, and high solar radiation, leading to low SOC- (corroborated the PCA analysis). The highest  $S$   
559 were found in sites SE7 and SE8, in which the former presented high clay and SOC content, and  
560 SE8 had high precipitation and low PET.

561 For the tillage system treatments in Sweden, similar to the Austrian sites, the conventional tillage  
562 presented the lowest  $k$ , and also lowest  $S$ . Even when comparing tillage systems in Sweden and  
563 Austria jointly (Table S3) we could notice that conventional tillage also presented the lowest  $k$ ,  
564 while ~~DRT~~deep reduced tillage the highest.

565 ~~The mean  $k$  was higher in Sweden, while the mean  $S$  was higher in Austria (Table 4, Fig. 4). In~~  
566 ~~general, the variation in  $k$  values were lower in Austria, while the variation in  $S$  were lower in~~  
567 ~~Sweden. It is possible that the ash correction, that was made for the Swedish but not the Austrian~~

568 sites, may partly explain this difference. Indeed, the average mass loss after 90 days at the Swedish  
569 sites for Green and Rooibos tea was higher with about 60 and 30%, respectively, whereas it was  
570 only about 45 and 15% for the Austrian sites (data not shown). When recovering litter dry matter  
571 from the soil, soil contamination are often not negligible. In our study, the ash content determined  
572 on the Green and Rooibos tea bags for the Swedish site represented  $15 \pm 6$  and  $10 \pm 4$  %, respectively  
573 (data not shown).

574  
575 The decomposition rate  $k$  was mostly affected by climatic conditions, which was already expected,  
576 as temperature and aridity or moisture, however agronomic and edaphic factors proved to be of  
577 great relevance for  $k$ , as soil management choice (treatment), soil C:N ratio, SOC, crop type, pH,  
578 clay content, soil nitrogen and others. *Influence of climate and soil properties*

579 ~~In previous studies using the TBI approach, it was shown that climate played a significant role on~~  
580 ~~decomposition in a temperate biome (Djukic et al., 2018), but when comparing several different~~  
581 ~~biomes, climatic conditions were of relatively low importance (Fanin et al., 2020). In Boreal soils,~~  
582 ~~Althuizen et al. (2018) found that increased temperatures enhanced  $k$ , whereas increased~~  
583 ~~precipitation decreased  $k$  across years. Despite that many studies have showed a positive~~  
584 ~~correlation between precipitation and decomposition rates (Pimentel et al., 2019; García Palacios~~  
585 ~~et al., 2016), precipitation did not have a huge impact in our study according the random forest~~  
586 ~~analysis. On the other hand,  $Re_{water}$  showed great importance (Fig. 6). It is because this variable~~  
587 ~~includes nonlinearities due to its shape according to which decomposition increases with soil~~  
588 ~~moisture and then decreases at high soil water content due to oxygen limitation of microorganisms~~  
589 ~~(Moyano et al., 2013).~~

590 In general, higher  $k$  values were observed when the aridity index (AI) was lower. AI was identified  
591 by the random forest regression model being an important variable affecting the rate of  
592 decomposition (Fig. 6). Soils from more arid and warmer sites are associated with lower SOC  
593 (Kerr and Ochsner, 2020; Ontl and Schulte, 2012). With increasing aridity, the biological processes  
594 that drive C and N inputs and fluxes in ecosystems may be impaired, which may result in  
595 decreasing soil C and N stocks (Jiao et al., 2016; Reynolds et al., 2007).

596 The random forest models showed that the decomposition rate  $k$  was mostly affected by climate,  
597 in particular when considering the TBI periods combined (Fig. 6a). The lower predictive power of  
598 the models when considering the 60 and the 90 days TBI periods separately can explain the higher  
599 number of variables considered, due to less defined effects to be identified by the model. This is  
600 suggested also by the decrease in the overall node purity of the models using only 60, or 90 days  
601 data to explain  $k$ . When using the combined dataset, the model was instead explaining a relatively  
602 large part of the variance ( $R^2=0.735$ ) and with a much higher node purity, while employing very  
603 few and only climatic related parameters.

604 For practical reasons the teabags in our study were buried in the soil during the growing season,  
605 corresponding to a period when the soil biological activity is highest (Bolinder et al., 2013). When  
606 burying the teabags during the growing season, the difference in climate between sites are  
607 attenuated, in particular with respect to air temperature. For example, the MAT at the Swedish  
608 most northerly (SE5 and SE9) and southerly (SE2) sites are 4.1 and 9.0 °C, respectively, whereas  
609 corresponding mean air temperatures ( $MAT_{TBI}$ ) during our study were 14.3 and 16.2 °C,  
610 respectively.

611 The TBI  $S$  parameter was also dependent on climate. Indeed, the random forest model identified  
612 climatic parameters as the main factors affecting  $S$  in both Austria and Sweden during all evaluated

613 ~~periods. In particular,  $Re_{clim}$  and  $Re_{temp}$  often showed significant negative correlations, which~~  
614 ~~implies a negative impact of air temperature on  $S$ . However, raw climatic variables, such as~~  
615 ~~precipitation and temperature were only weakly correlated with  $S$ . This is probably due to~~  
616 ~~nonlinear processes, which are considered in the ICBM climate dependent soil biological activity~~  
617 ~~calculations such as  $Re_{wet}$  (as discussed above). Furthermore, since litter decomposition dynamics~~  
618 ~~is influenced by multiple factors that interact and change over time (Bradford et al., 2016), the~~  
619 ~~relationships are not always linear. Random forest models that we fitted to the data are more~~  
620 ~~efficient capturing such combinations and interactions of factors, and can detect relationships that~~  
621 ~~would not be detectable by linear approaches.~~

622 The stabilization factor  $S$  expresses the degree by which the labile fraction of the plant material is  
623 decomposed. ~~Therefore, it is not surprising~~We observed that ~~more variables come into play to~~  
624 ~~define it. In particular, we noticed~~ the influence of ~~edaphic factors, of which~~ pH was the most  
625 important, ~~but also SOC concentration, C:N ratio~~ edaphic factor, and ~~clay were also identified as~~  
626 ~~good predictors. In addition, the~~ agronomic factors ~~were also influencing  $S$ , whereas the~~ soil  
627 management treatment, crop type, and ~~crop types were the most important. N fertilizer application~~  
628 were also identified as good predictors. A study conducted by Fu et al. (2021) suggested that pH,  
629 nutrient availability and soil compaction were the main reasons contributing to the differences in  
630 litter decomposition. The net effect of pH is not clear since it modifies both SOC decay kinetics  
631 and productivity simultaneously (Paradelo et al., 2015). ~~Nevertheless, the impact of pH on SOC~~  
632 ~~kinetics seems clear in our study~~, with a maximum effect at around neutral pH (Liao et al., 2016).  
633 Nevertheless, the impact of pH on litter decomposition using the TBI approach seems clear in our  
634 study.

635

636

## 637 **Conclusion**

638 Our results show that both the TBI  $k$  and  $S$  parameters were sensitive to management practices in  
639 agroecosystems in Austria and Sweden. We were observing significant differences for some of the  
640 treatments in all categories of LTEs. Notably, for the effect of crop residue incorporation, organic  
641 amendments and N ~~fertilization~~fertilizer application, crop types and tillage systems. In the  
642 Austrian LTEs, application of green manure + municipal compost showed a higher  $S$  compared to  
643 the application of other organic amendments. Incorporation of crop residues and high N  
644 ~~fertilization~~fertilizer application also increased  $k$ . In the Swedish LTEs, it was shown that  
645 combined management practices with both farmyard manure and mineral NPK resulted in higher  
646  $k$  and  $S$  compared to no manure and no NPK applications, whereas growing spring cereals instead  
647 of leys increased  $k$  but did not change  $S$ . For both countries, tillage systems with deep reduced  
648 tillage practices presented higher  $k$ , and shallow reduced tillage presented higher  $S$ . However, these  
649 effects were also site or year dependent within a given country. Climatic conditions had the most  
650 important impact on the decomposition rate  $k$  and the stabilization factor  $S$ , but also pH, treatment,  
651 crop types, SOC, C:N ratio and clay content were good predictors of the TBI parameters.  
652 Generally, the correlations with raw climatic variables such as precipitation and temperature were  
653 quite poor. Better relationships were found when nonlinearities due to interactions between  
654 climatic and edaphic conditions were accounted for. Our results ~~bring knowledge and answers~~  
655 ~~under~~highlights how a wide range of soil management practices affect ~~soil decomposition-TBI~~  
656 ~~parameters~~ jointly to soil and climatic conditions. ~~We recommend the TBI approach for further~~  
657 ~~LTE studies evaluating soil decomposition dynamics.~~

658

## 659 **Data availability**

660 Data can be provided by the authors upon request.

661

## 662 **Author contribution**

663 MRG, MB and TS wrote and prepared the manuscript draft; MB, TK and TS supervised and led  
664 the research; MB, OA, HS, JKF, AS, AS and TS developed the methodology; MRG and LM  
665 analyzed the data. All authors reviewed and edited the manuscript.

666

## 667 **Competing interests**

668 The contact author has declared that none of the authors has any competing interests.

669

## 670 **Acknowledgements**

671 Financial support was provided by the Swedish Farmers' Foundation for Agricultural Research,  
672 grant number O-18-23-141. Part of this research has been done in the framework of the EJP SOIL  
673 that has received funding from the European Union's Horizon 2020 research and innovation  
674 programme: Grant agreement No 862695.

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958

959 **Tables**

960

961 Table 1. Experimental sites in Austria. Site acronym, location name, year of TBI measurements,  
 962 category and type of LTE including its duration, main crop cultivated during the TBI  
 963 measurements and management treatments

964

Site	Location	Year	Category*	Experiment <sup>†</sup>	Age	Crop	Treatments <sup>§</sup>
AT1	MUBIL	2014	CB	OA	11	Luzerne	GM, FW, FYM, BS
AT2	MUBIL	2015	CB	OA	12	Wheat	GM, FW, FYM, BS
AT3	Ritzlhof	2015	CB	IF-N & OA+N	24	Maize	0, 40, 90, 120 N CFW, CGM, CS, CSS+80N
AT4	Rottenhaus	2016	CB	CR with NPK	31	Maize	CRR, CRI
AT5	Rutzendorf	2015	CB	CR with NPK	34	Wheat	CRR, CRI
AT6	Rutzendorf	2016	CB	CR with NPK	35	Maize	CRR, CRI
AT7	Breitstetten	2015	SF	IF-N with PK	40	Wheat	60, 90, 120, 145 N
AT8	Breitstetten	2016	SF	IF-N with PK	41	Wheat	60, 90, 120, 145 N
AT9	Haringsee	2015	SF	IF-N with PK	40	Wheat	60, 90, 120, 145 N
AT10	Fuchsenbigl	2016	SF	IF-K with NP	61	Maize	0, 150, 300 K
AT11	Rottenhaus	2016	SF	IF-K with NP	63	Maize	0, 150, 300 K
AT12	Haringsee	2016	SF	IF-N without PK	41	Maize	0, 60, 120, 180 N
AT13	Zinsenhof	2016	SF	IF-N without PK	41	Maize	0, 60, 120, 180 N
AT14	Zissersdorf	2016	SF	IF-N without PK	41	Maize	0, 60, 120, 180 N
AT15	Fuchsenbigl	2015	TS	TS	27	Maize	CT, SRT, DRT
AT16	Fuchsenbigl	2016	TS	TS	28	Wheat	CT, SRT, DRT

965 \* CB: carbon balance practices; SF: soil fertility; TS: tillage systems.

966 † OA: organic amendments; IF-N & OA+N: N inorganic fertilization and organic amendments plus mineral N; CR &  
 967 IF-P with NK: crop residues and P inorganic fertilization with 90 and 40 kg ha<sup>-1</sup> of N and K, respectively; IF-N with  
 968 PK: N inorganic fertilization with 55 and 180 kg ha<sup>-1</sup> of P and K, respectively; IF-N without PK: N inorganic  
 969 fertilization; IF-K with NP: K inorganic fertilization with 40 and 120 kg N-ha<sup>-1</sup> of P and K; TS: tillage system.

970 § GM: green manure; FW: municipal compost and green manure; FYM: farmyard manure; BS: biogas slurry; CFW:  
 971 compost food waste with 175 kg N ha<sup>-1</sup>; CGM: compost green manure with 175 kg N ha<sup>-1</sup>; CS: compost slurry with  
 972 175 kg N ha<sup>-1</sup>; CSS: compost sewage sludge with 175 kg N ha<sup>-1</sup>; CRR: crop residues removed; CRI: crop residues  
 973 incorporated; CT: conventional tillage; SRT: shallow reduced tillage; DRT: deep reduced tillage.

974

975



976 Table 2. Experimental sites in Sweden. Site acronym, location name, year of TBI measurements  
 977 conducted in 2016, type of LTE including its duration, main crop cultivated during the TBI  
 978 measurements and management treatments  
 979

Site	Name	Experiment*	Age	Crop	Treatments†
SE1	Börgeby	CMP	59	Sugar beet	FYM/NPK
				Sugar beet	0 FYM/0 NPK
SE2	Ekebo	CMP	59	Sugar beet	FYM/NPK
				Sugar beet	0 FYM/0 NPK
SE3	Högåsa	CMP	50	Ley production year	FYM/NPK
				Ley production year	FYM/0 NPK
				Spring oilseed	0 FYM/NPK
				Spring oilseed	0 FYM/0 NPK
SE4	Kungsängen	CMP	53	Oat	FYM/NPK
				Oat	0 FYM/0 NPK
SE5	Röbacksdalen	CMP	47	Barley	FYM/NPK
				Barley	FYM/0 NPK
SE6	Vreta Kloster	CMP	50	Ley production year	FYM/NPK
				Ley production year	FYM/0 NPK
				Spring oilseed	0 FYM/NPK
				Spring oilseed	0 FYM/0 NPK
SE7	Lanna	ROT	35	Oat	SC
				Ley production year	L
SE8	Lönstorp	ROT	36	Barley	
				Ley	SC
				<del>establishment</del> establish	L
				1 year	
SE9	Röbacksdalen	ROT	36	Barley	
				Ley	SC
				<del>establishment</del> establish	L
				1 year	
SE10	Säby	ROT	46	Wheat	
				Ley	SC
				<del>establishment</del> establish	L
				1 year	
SE11	Lanna	TS	34	Winter wheat	CT, DS
SE12	Säby	TS	11	Barley	CT, SRT, DRT, DS
SE13	Ultuna	TS	19	Barley	CT, DRT

980  
 981 \* CMP: combined management practices; ROT: rotation systems; TS: tillage systems.  
 982 † FYM/NPK: maximum amount of farmyard manure and maximum doses of NPK; 0 FYM/0 NPK: no manure and no  
 983 NPK application; FYM/0 NPK: maximum amount of farmyard manure and no NPK application; 0 FYM/NPK: no  
 984 manure and maximum doses of NPK; SC: spring cereal; L: ley; CT: conventional tillage; DS: direct seeding; SRT:  
 985 shallow reduced tillage; DRT: deep reduced tillage.  
 986



988 Table 3. Annual climatic characteristics for the Austrian and Swedish sites during the entire year  
 989 of measurements and only during the TBI period (days) corresponding to the period between the  
 990 date of placement and the last retrieval date of the tea bags.  
 991

Site	TBI period	TAP	TP <sub>TBI</sub>	MAT	MAT <sub>TBI</sub>	PET	PET <sub>TBI</sub>	AI	AI <sub>TBI</sub>
	days	mm		°C		mm			
<i>Austria</i>									
AT1	99	756	294	12.5	20.3	811.4	414.0	1.07	1.4
AT2	61	516	65	12.6	18.8	605.1	141.3	1.17	2.2
AT3	80	622	161	12.2	20.0	682.9	295.1	1.09	1.8
AT4 & 11	84	939	384	11.2	20.5	763.4	306.1	0.81	0.8
AT5	63	516	82	12.6	19.4	605.1	148.6	1.17	1.8
AT6	96	735	226	12.0	21.6	854.2	394.0	1.16	1.7
AT7	59	516	81	12.6	19.3	605.1	137.4	1.17	1.7
AT8	84	735	240	12.0	17.1	854.2	334.2	1.16	1.4
AT9	60	516	81	12.6	19.2	605.1	139.6	1.17	1.7
AT10 & 16	92	735	205	12.0	21.4	851.2	378.9	1.15	1.8
AT12	87	735	203	12.0	21.8	854.2	367.4	1.16	1.8
AT13	84	939	327	11.2	20.5	763.4	304.3	0.81	0.9
AT14	82	735	154	12.0	21.3	854.2	319.6	1.16	2.1
AT15	81	516	103	12.6	22.5	605.1	212.9	1.17	2.1
<i>Sweden</i>									
SE1	92	436	141	9.2	16.7	444.6	235.1	1.02	1.7
SE2	91	681	270	9.0	16.5	578.5	297.8	0.85	1.1
SE3	91	458	142	7.9	13.3	618.0	306.4	1.35	2.1
SE4	93	429	126	7.1	15.3	640.0	367.0	1.48	2.9
SE5 & 9	92	526	164	4.1	14.3	396.2	218.2	0.75	1.3
SE6	91	433	158	7.9	13.3	617.5	301.8	1.16	1.9
SE7 & 11	91	412	115	7.4	12.7	514.2	276.4	1.3	2.4
SE8	90	610	224	9.4	14.4	500.2	265.7	0.82	1.2
SE10 & 12	91	429	126	7.1	15.3	358.5	155.7	0.84	1.2
SE13	92	429	126	7.1	15.3	639.1	377.8	1.5	3.0

992  
 993 TAP: total annual precipitation; TP<sub>TBI</sub>: total precipitation during TBI period; MAT: mean annual temperature;  
 994 MAT<sub>TBI</sub>: mean temperature during TBI period; PET: total annual potential evapotranspiration; PET<sub>TBI</sub>: potential  
 995 evapotranspiration during TBI period; AI: annual aridity index (PET divided by TAP); AI<sub>TBI</sub>: aridity index during TBI  
 996 period.  
 997

998

999 Table 4 – Mean values of decomposition rate ( $k$ ) and stabilization factor ( $S$ ) for the TBI approach  
 1000 after ~~60 and 90 days of the~~ incubation period.

1001

Incubation	Mean TBI parameters			
	$k$	$S$		
			<i>Sweden</i>	
			<del>60 days</del>	<del>0.0160</del>
<del>90 days</del>	<del>0.0160 ± 0.01</del>	<del>0.247 ± 0.14</del>		
<i>Sweden</i>			<i>Austria</i>	
			<del>60 days</del>	<del>0.0152</del>
<del>90 days</del>	<del>0.0115 ± 0.004</del>	<del>0.228 ± 0.11</del>		<del>0.289</del>
<i>Austria</i>				

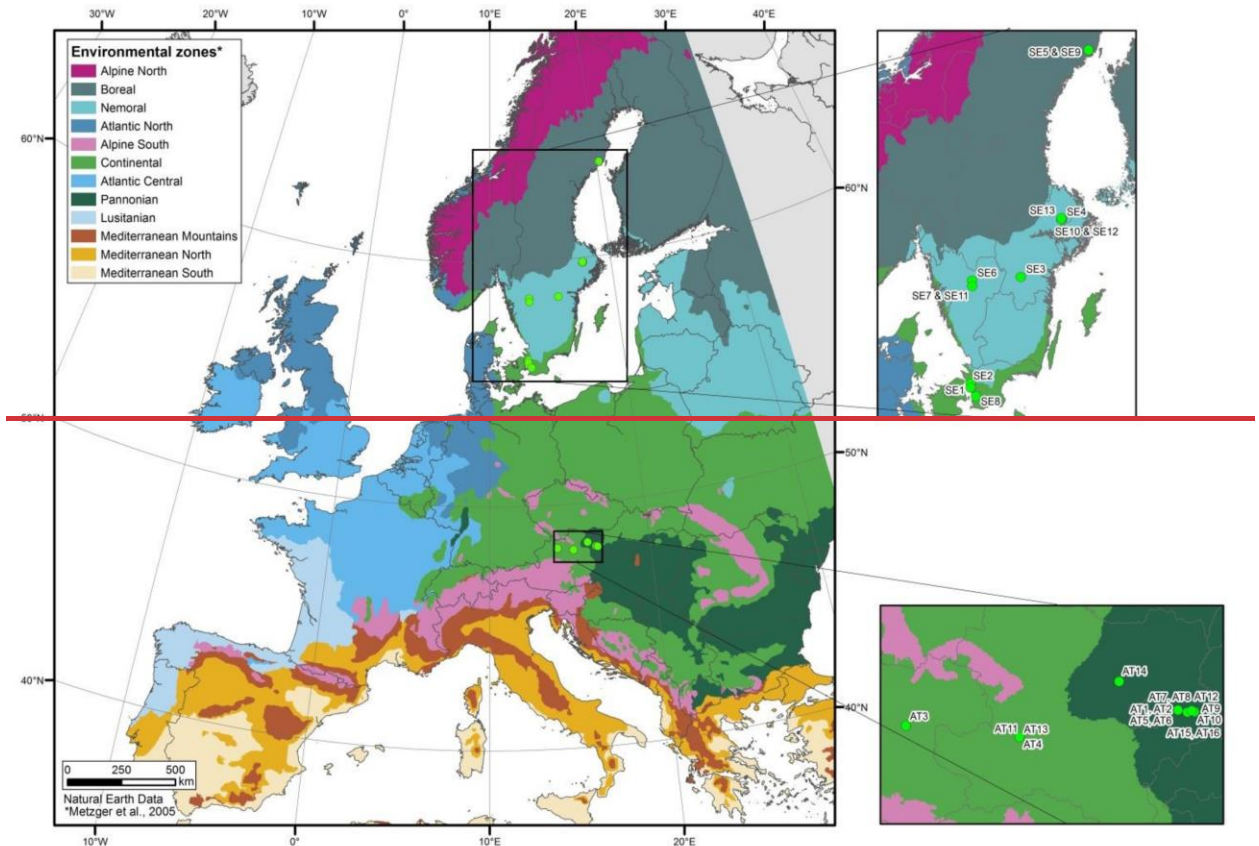
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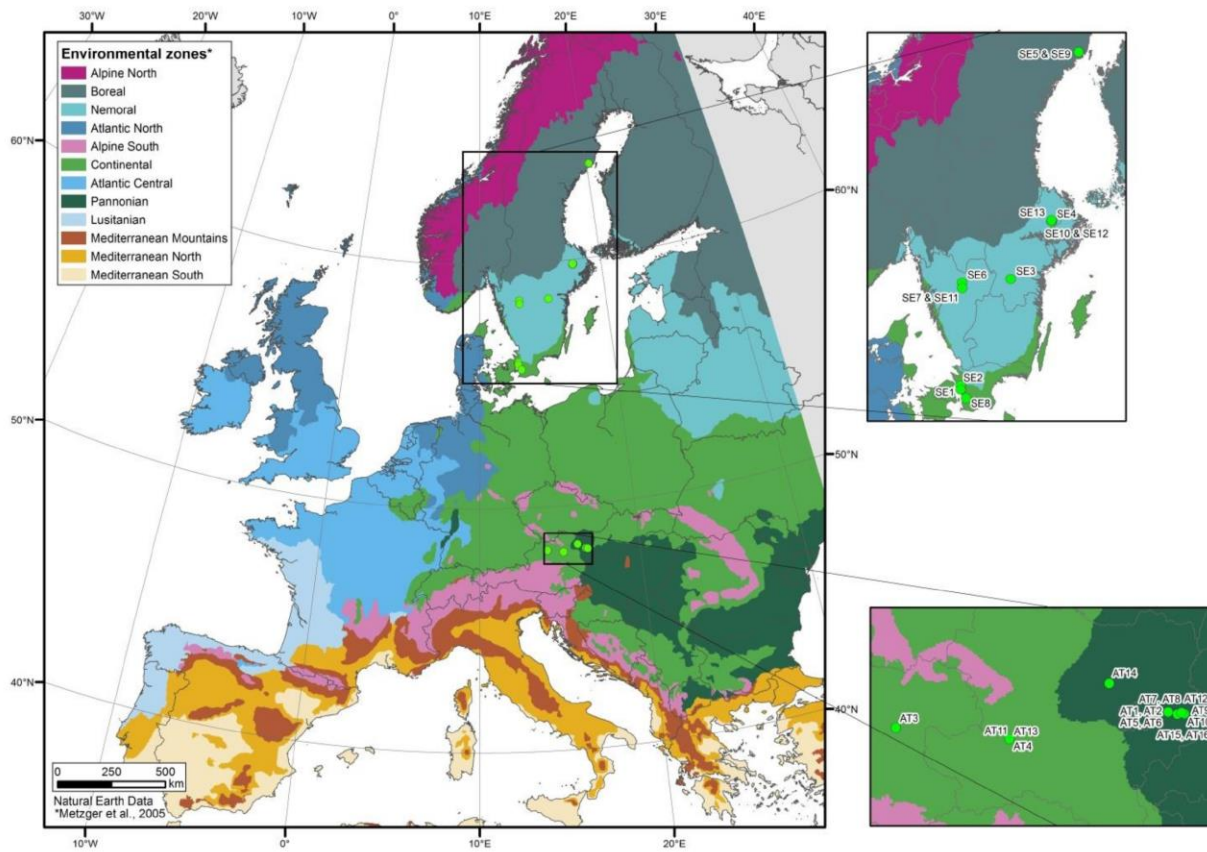
1004 **Figures**

1005

1006 **FIGURE 1**



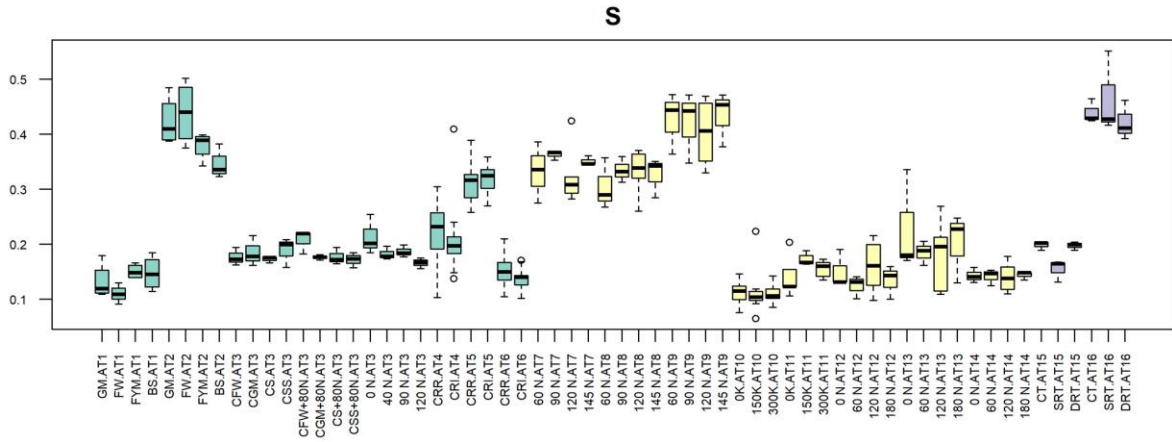
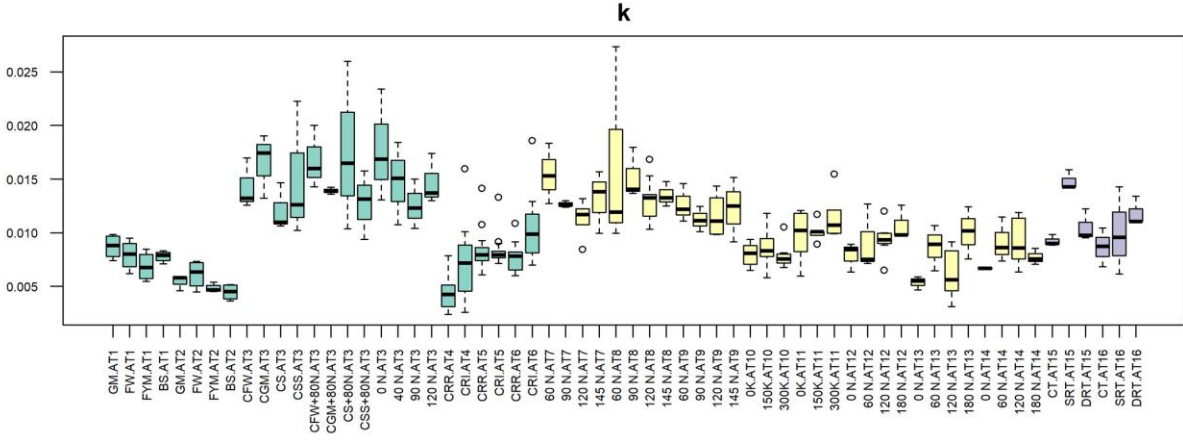
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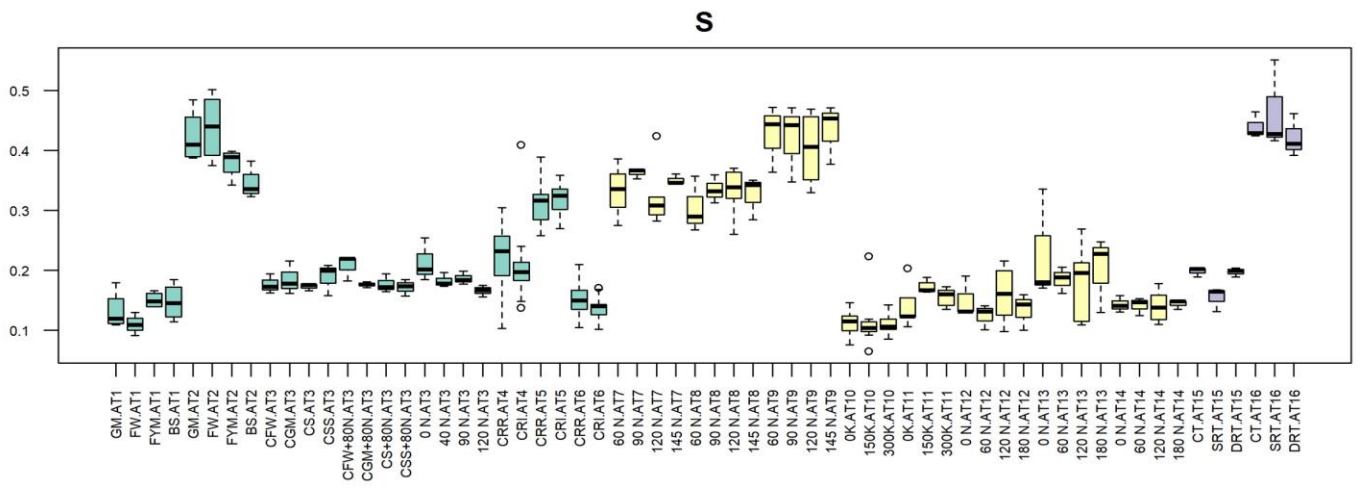
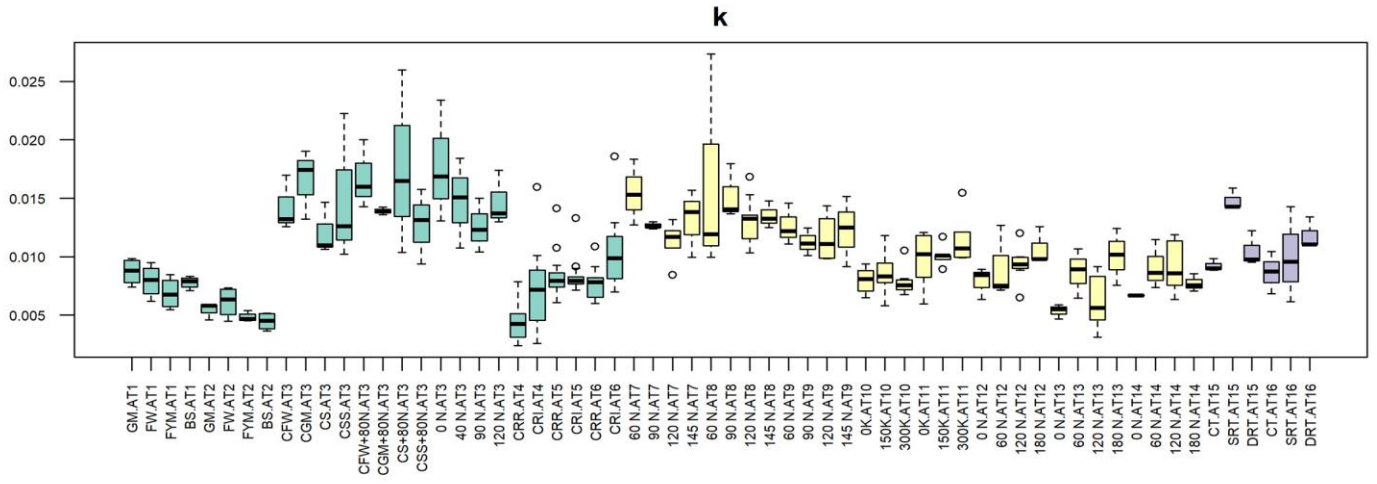


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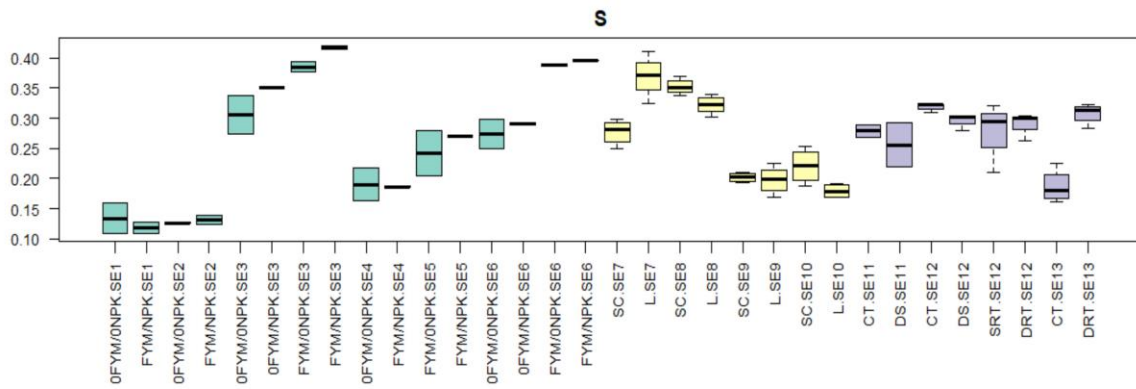
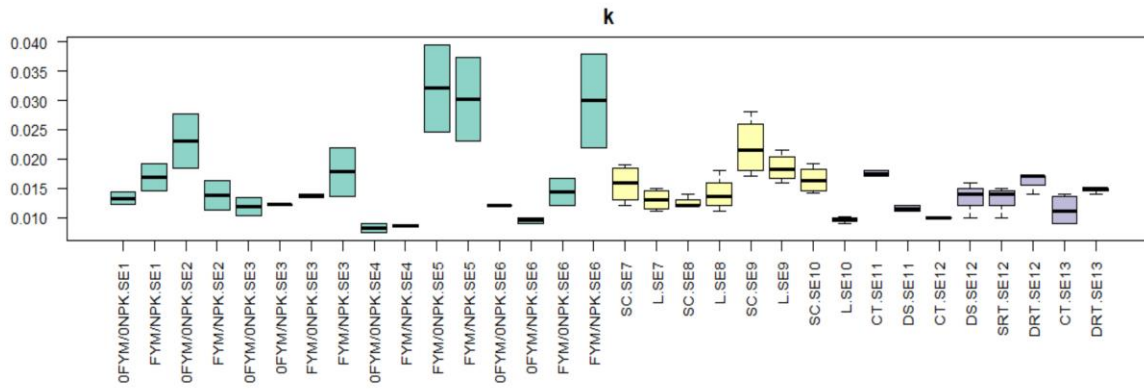


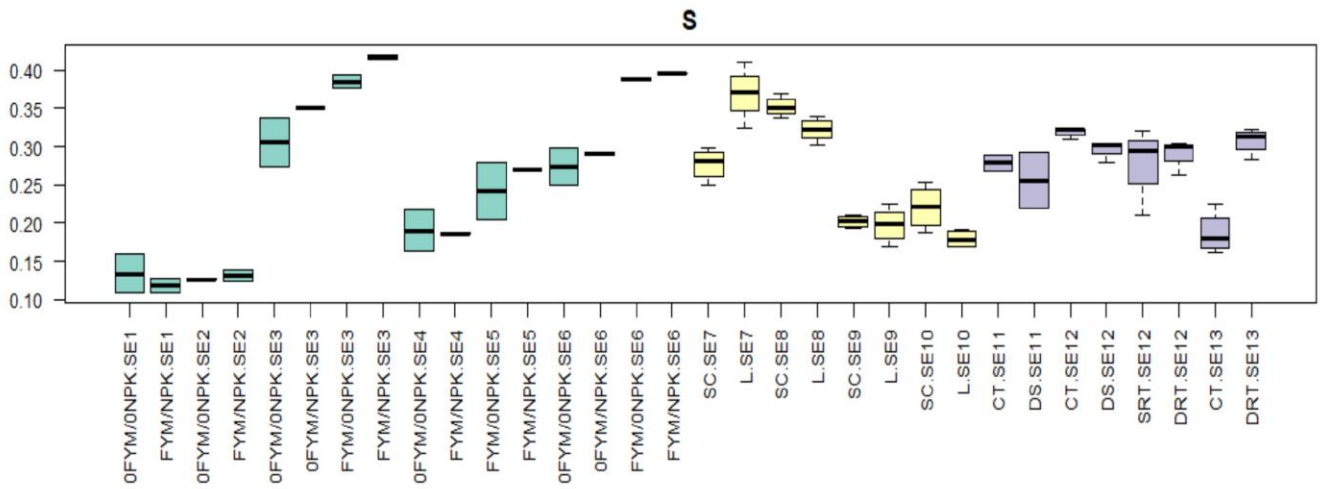
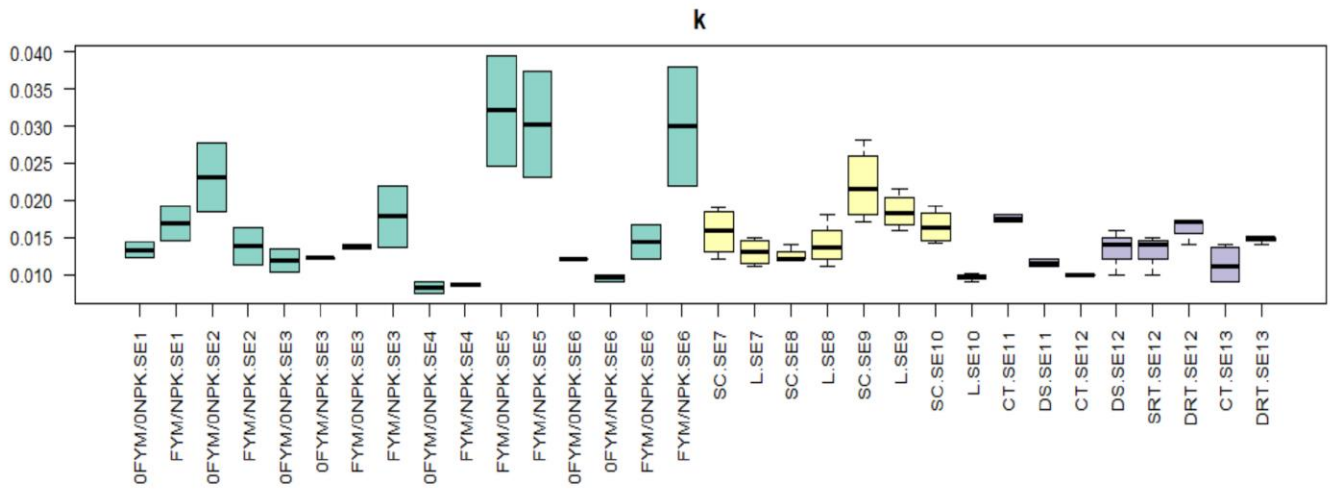




1012 **FIGURE 3**

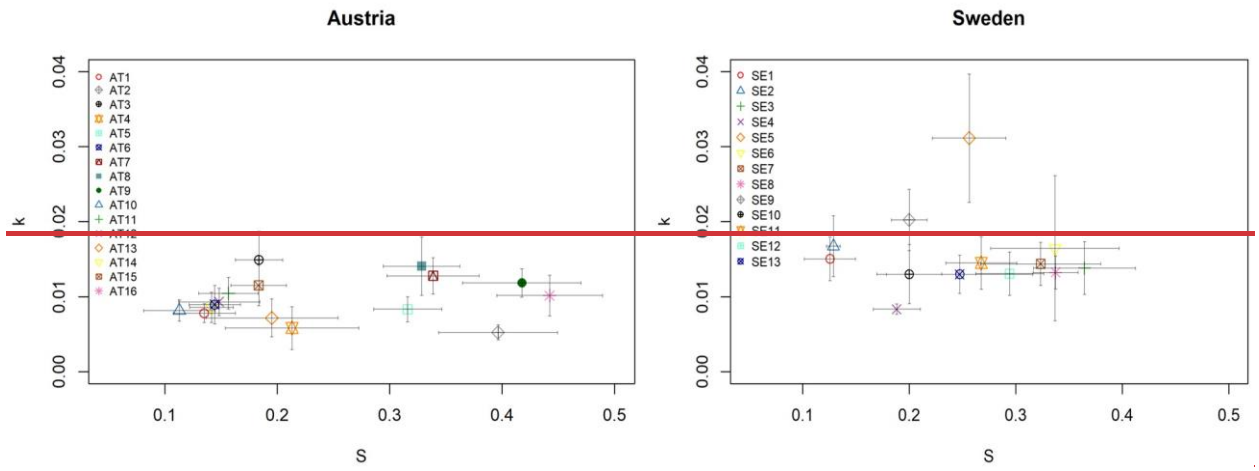
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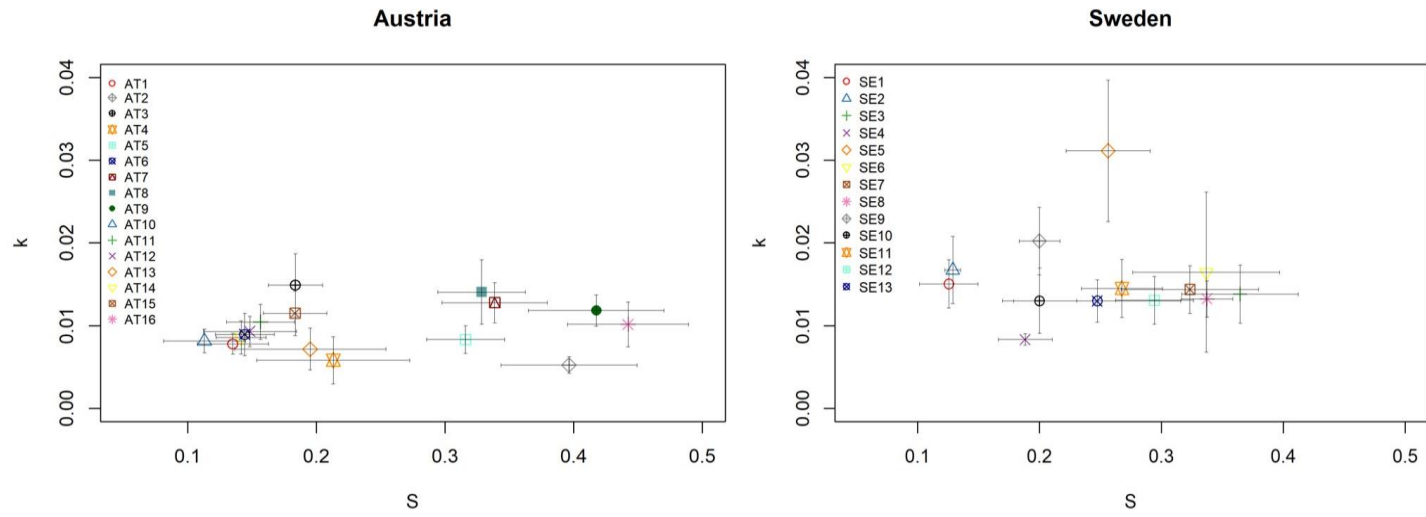


1016 **FIGURE 4**

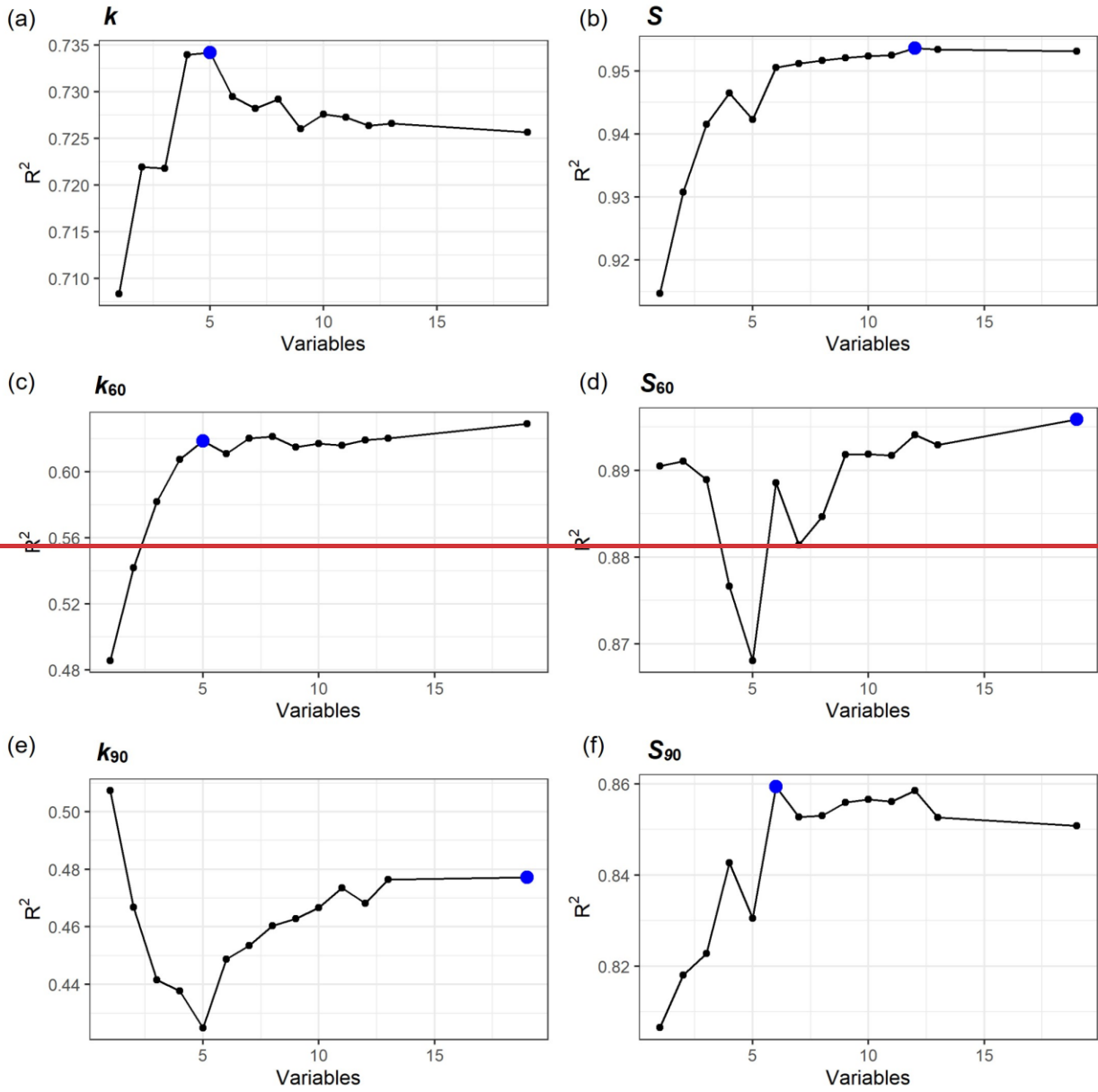
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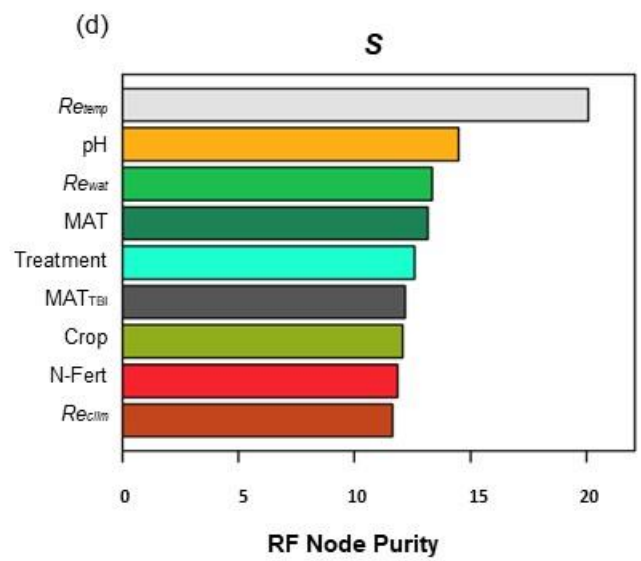
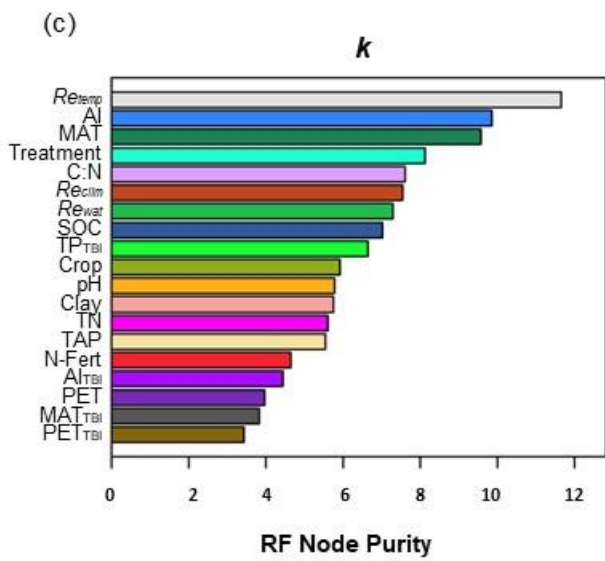
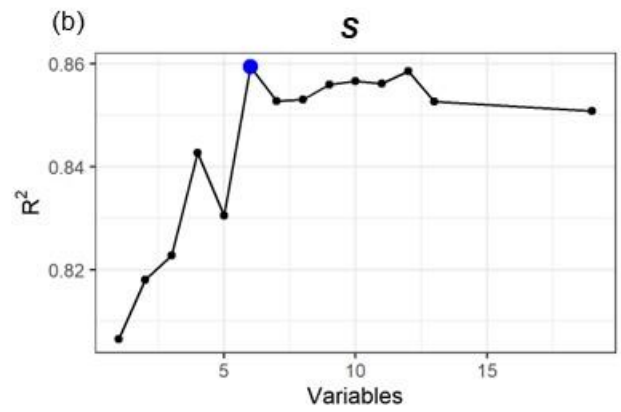
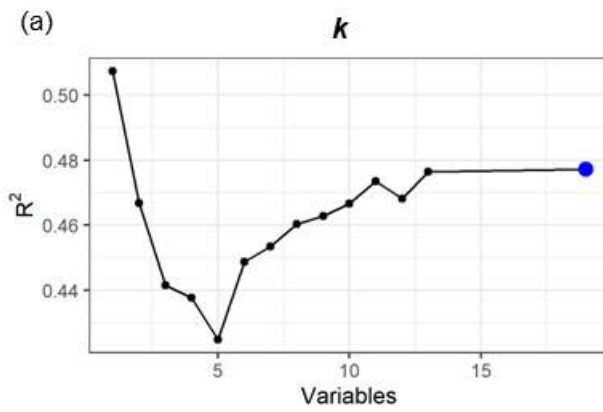


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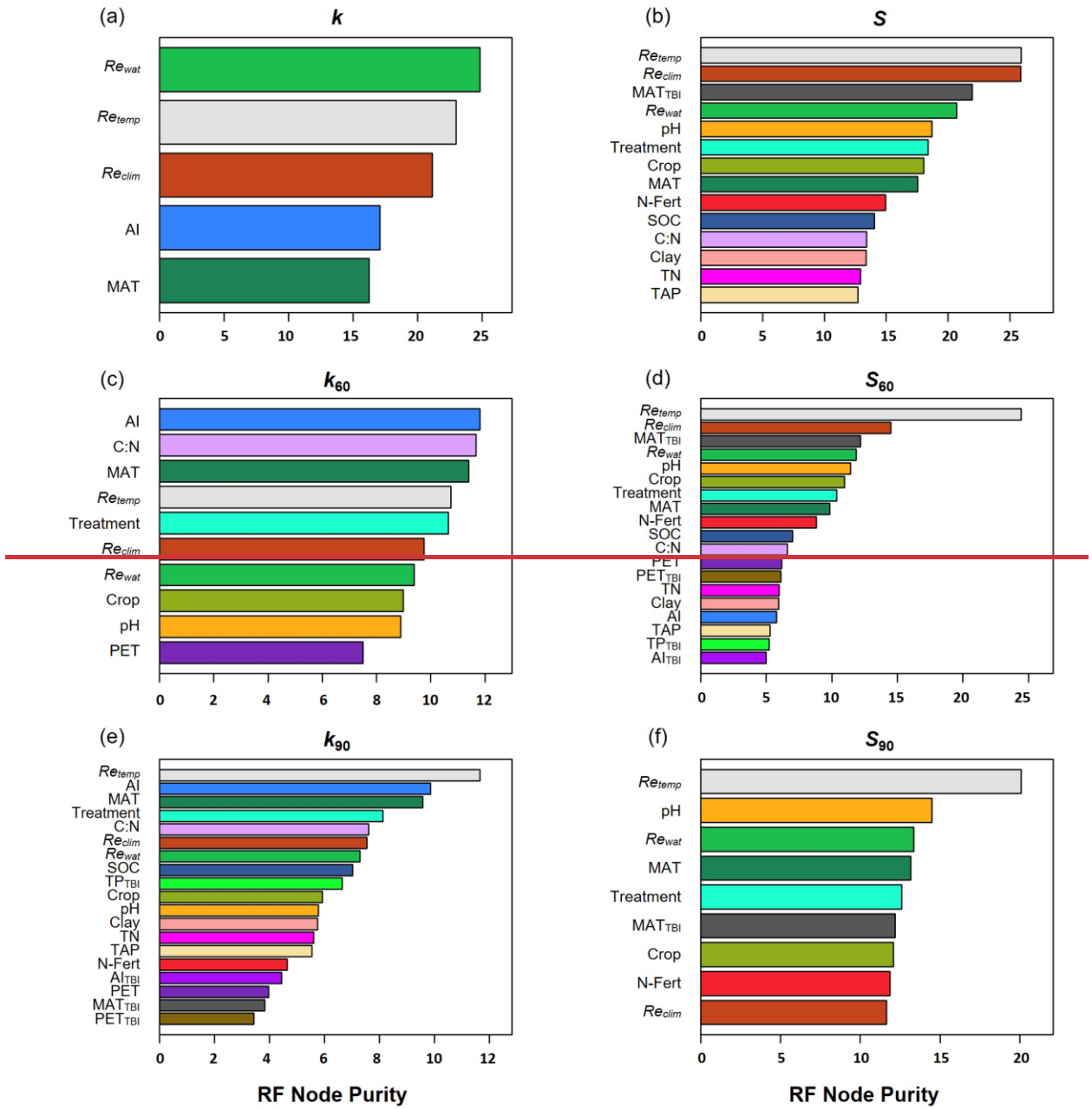


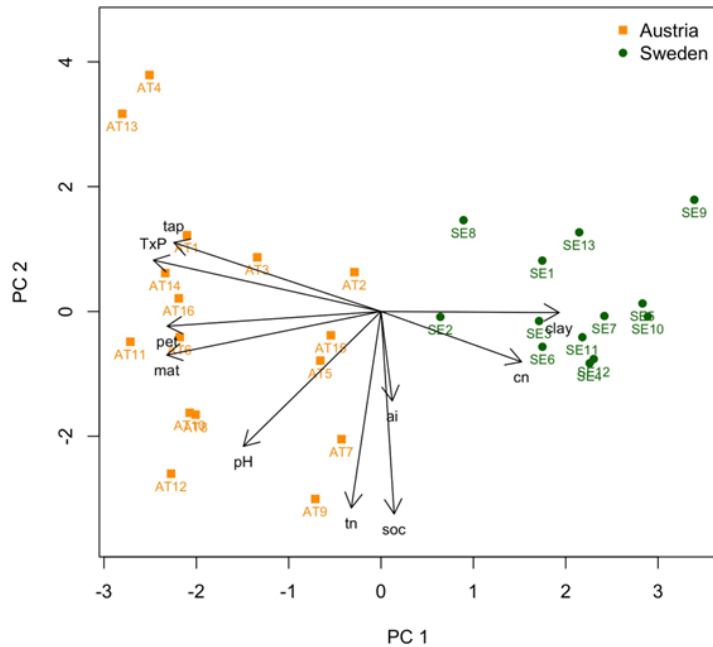
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1029

1030

1031 **Figure caption**

1032

1033 Figure 1 - Location and environmental zone of the Austrian and Swedish sites.

1034

1035 Figure 2 - Average decomposition rate ( $k$ ) and stabilization factor ( $S$ ) after the 90 days TBI period

1036 for each treatment and site in Austria. The extents of the box indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and

1037 the lines represent the 50<sup>th</sup> percentile. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles and outliers

1038 are given as open symbols. Green boxes: Carbon balance (CB) experiment; yellow boxes: Soil

1039 fertility (SF) experiment; purple boxes: tillage systems (TS) experiment. Site AT1 shows results

1040 from 2014. Sites AT2, AT3, AT5, AT7, AT9, and AT15 show results from 2015. And sites AT4,

1041 AT6, AT8, AT10, AT11, AT12, AT13, AT14, and AT16 show results from 2016.

1042

1043 Figure 3 - Average decomposition rate ( $k$ ) and stabilization ( ~~$S$  after the 90 days TBI period~~factor  
1044 ( $S$ ) for each site and treatment in Sweden. The extents of the box indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles,  
1045 and the lines represent the 50<sup>th</sup> percentile. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Green  
1046 boxes: combined management practices (CMP) experiment; yellow boxes: rotation (ROT)  
1047 experiment; purple boxes: tillage systems (TS) experiment.

1048  
1049 Figure 4 - Distribution of the mean decomposition rate constant ( $k$ ) and the stabilization factor ( $S$ )  
1050 ~~after the 90 days TBI period~~ for each site in Austria and Sweden. Errors bars represent standard  
1051 deviation.

1052  
1053 Figure 5 - a and b) Variables selection procedure to identify the optimal number of variables to  
1054 explain the variance of  $k$  and  $S$  ~~considering the combined dataset (60 and 90 days TBI period)~~ with  
1055 a Random Forest model. The blue point represents the optimal model. ~~a) and b) Variables affecting~~  
1056  ~~$k$  and  $S$  over all sampling times; c) c and d) Variables affecting  $k$  and  $S$  after 60 days; e) and f)~~  
1057 ~~Variables affecting  $k$  and  $S$  after 90 days.~~

1058  
1059 ~~Figure 6~~ - Relative importance of the variables used by each optimized Random Forest model to  
1060 predict the variance in the  $k$  and  $S$  parameters ~~for the combined dataset (60 and 90 days TBI period)~~  
1061 in Austria and Sweden jointly. The higher the Node purity, the higher the importance of such  
1062 variable. ~~a)~~

1063  
1064 Figure 6 - Principal component analysis showing how the sites in Austria and Sweden differ based  
1065 on the variables. PC1 and PC2 are the first two components, explaining most variance. The

1066 loadings (black arrows) are the weight of each variable in defining each principal component. The  
1067 size of the arrows can tell how much they contribute defining this space, while the direction is their  
1068 contribution on each axis. Tap: total annual precipitation; TxP: temperature x precipitation factor;  
1069 pet: potential evapotranspiration; mat: mean annual temperature; tn: total soil nitrogen; soc: total  
1070 soil organic carbon; cn: soil C:N ratio; ai: aridity index.

1071 ~~b) Variables affecting  $k$  and  $S$  in all times; c) and d) Variables affecting  $k$  and  $S$  after 60 days; e)~~  
1072 ~~and f) Variables affecting  $k$  and  $S$  after 90 days.~~