

1 **Teatime for *Triticum* – (how) can the presence of plants slow** 2 **down decomposition?**

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19 **Abstract**

20 Decomposition of organic matter is a key process in soils contributing to carbon and nutrient
21 cycling. To identify management strategies for agroecosystems that reduce nutrient losses
22 while maximizing plant growth, it is important to understand which parameters determine
23 decomposition rates. This study therefore investigated how the presence of winter wheat
24 (*Triticum aestivum* var. Asory) affects decomposition in a controlled Ecotron setup with two
25 soil types with varying organic matter content across three simulated climates (2013, 2068,
26 2085). Using the tea bag index, interstitial soil pore water analyses, microbial biomass
27 quantification, bacterial and fungal gene abundance, and soil respiration measurements, we
28 tested the hypotheses that plant exudates would enhance decomposition rate and microbial
29 biomass, while plant nitrogen uptake would deplete soil nitrate, potentially mitigated by
30 fertilization. Contrary to expectations, decomposition rates were lower in planted than in
31 unplanted soils, suggesting resource competition between plants and microbes. No significant
32 differences were observed in microbial biomass or respiration due to plant presence, and
33 fertilization effects on nitrate or microbial mineralization were undetectable, likely due to
34 rapid turnover of organic molecules including uptake by plants and microbes.
35 Mechanistically, fungi and soil humidity were more important for decomposition than
36 bacteria or temperature. The findings corroborate climate impacts on decomposition but also
37 indicate microbial resilience and highlight the potential of management strategies like cover
38 crops, adjusted planting dates and crop residual management which can contribute to healthy
39 soils by sustaining carbon and nutrient cycling.

40 1. Introduction

41 Decomposition is a fundamental ecosystem process that regulates carbon and nutrient
 42 cycling, influencing soil fertility and plant productivity in agricultural systems. In the context
 43 of climate change, understanding how biotic factors, such as the presence of crop plants,
 44 interact with abiotic conditions to modulate decomposition is essential for sustainable
 45 farming. Plants can potentially accelerate decomposition through root exudates, particularly
 46 carbohydrate-rich compounds that fuel microbial activity and mineralization^[1,2,3,4].
 47 Conversely, plants may compete with microbes for resources like nitrogen, slowing
 48 decomposition via nutrient limitation^[5,6,7,8]. This duality is particularly relevant for crops like
 49 winter wheat (*Triticum aestivum*), where effective nutrient management may determine how
 50 yields evolve under future climate^[9,10].

51 Despite extensive research on decomposition, few studies have focused explicitly on the
 52 effect of presence or absence of living plants, despite the relevance for agricultural land
 53 management. The plant effect has been studied even less under predicted future
 54 meteorological conditions, a critical research gap as projected climate scenarios with warmer
 55 temperatures and variable precipitation could exacerbate or buffer nutrient losses, depending
 56 on microbial communities and soil organic matter (SOM) content, which affects microbial
 57 resilience and water retention^[11,12,13]. Here, we test how winter wheat presence influences
 58 decomposition, microbial biomass, bacterial and fungal gene abundance, respiration, and
 59 glucose and nitrate dynamics in presence or absence of plants based on an Ecotron
 60 experiment. The Ecotron experiment exposed cropping systems to the meteorological
 61 conditions of three different years across a climate gradient representative of climate change
 62 in the 21st century in Central Europe and used two contrasting soil types (low vs. high SOM).
 63 We hypothesized that plants enhance decomposition via exudates but deplete nitrate through
 64 uptake, with fertilization compensating for the latter. These insights aim to inform adaptive
 65 agricultural practices, emphasizing the role of plant-microbe interactions in maintaining soil
 66 health.

67 2. Material & methods

68 2.1 Hypotheses

69 This experiment focused on decomposition and nitrate availability in relation to the
 70 presence/absence of plants and in relation to nitrogen fertilization.

71 The first set of hypotheses was based on the premise that plants exude carbohydrate-rich
 72 substances which increase microbial mineralization^[1,2]. From this we deduced that

- 73 HP1a: Decomposition (tea bag index) is higher in planted than in unplanted cubes.
- 74 HP1b: Glucose-equivalents are higher in planted than in unplanted cubes.
- 75 HP1c: Microbial biomass (carbon, bacteria & fungi) is higher in planted than in unplanted
- 76 cubes.
- 77 HP1d: Soil-emitted CO₂ is higher in planted than in unplanted cubes.

78 The second set of hypotheses was based on the premises that plants take up nitrogen from the
 79 soil and that adding mineral nitrogen fertilizer would compensate plant N-uptake. We
 80 expected that the effect of fertilizer addition and plant presence would be visible in soil NO₃⁻
 81 concentrations and hypothesized:

82 HP2a: +fertilizer $\rightarrow$ NO $\square$ $\uparrow$

83 HP2b: +plant $\rightarrow$ NO $\square$ $\downarrow$

84 2.2 Experimental set-up

85 The experiment was conducted in the TERRA-Ecotron, where winter wheat (*Triticum*
86 *aestivum* var. Asory) was grown in the meteorological conditions of the years 2013, 2068,
87 and 2085, representing a gradient of present and predicted climate change scenarios for
88 Central Europe^[10]. As a second factor, the experiment used two soil types excavated from
89 real farmlands representing historically low (S1) vs. high (S2) soil organic matter inputs (10
90 vs 22 g SOC kg⁻¹ soil respectively, both C:N=10.5). The TERRA-Ecotron has six controlled
91 environment rooms (CERs), in each of which nine cubes (125L) with intact soil monolith can
92 be placed^[14]. In each CER, eight soil monoliths were planted and one was left unplanted.
93 Given that two soil types were used, there was one unplanted cube per soil type per climate.
94 In each CER, one cube with plants and the one existing cube without plants were selected for
95 the here presented decomposition study, covering all six soil x climate modalities in planted
96 and unplanted state. For the main study factor of this experiment, the presence or absence of
97 plants, there were thence n=6 replicates of each modality (plant yes/no), comprising the two
98 soils and three climates, which were not replicated within the plant treatment. In each of these
99 cubes, five bags of each tea type (green tea and rooibos tea) were placed at a depth of 8cm
100 (supplementary material SM0), leading to a total of n=120 tea bags being deployed. The
101 study started at the end of winter in 2068 and lasted from DAS131-DAS159. During this
102 time, the wheat was at the beginning, middle and end of tillering in 2013, 2068, and 2085
103 respectively (approx. BBCH 16,27,38). Only 2085 had received mineral fertilizer at the time
104 of study (Figure 1).

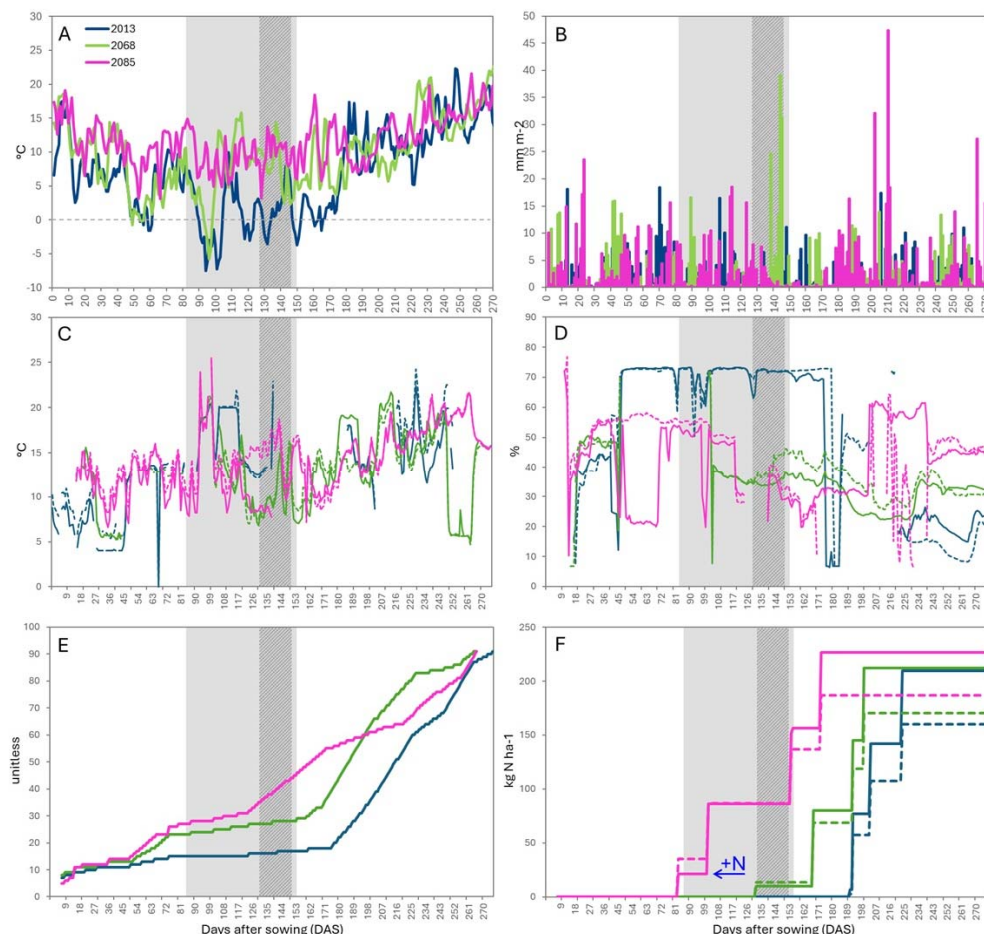


Figure 1: Environmental and soil conditions during the experiment. The study period is shaded in grey in each

plot, with the tea bag deployment period (DAS131-145) in dark dashed grey. If individual measures were performed for the two soil types S1 (low OM) and S2 (high OM), S1 is plotted with a straight line and S2 is plotted with a dashed line. A: Daily mean air temperature (°C), B: Daily cumulative precipitation (mm m⁻²), C: Daily soil temperature (°C), D: Daily mean volumetric soil humidity (%), E: Plant growth stages (BBCH), F: Cumulative nitrogen input from mineral fertilizer (kg N ha⁻¹). Note: N was applied in three doses according to phenology. Only 2085 had received N at the time of study (at DAS 102), with respective N-application indicated as “+N” in panel F.

2.3 Soil and climate conditions during the experiment

The experiment used two soil types of identical soil classification (silt loam (Aba(b)0)) but S2 had received twice as much organic matter than S1, resulting in 2x higher carbon and nutrient contents in S2 at similar C:N and pH (see [10] for detailed soil description). During the tea bag incubation (dark grey area in Figure 1), the air temperature followed the chronological sequence 2013<2068<2085, which also applied to soil temperature where it was measurable 2068<2085, with S2 being warmer than S1 (Table 1). Precipitations followed an inverse chronological pattern of 2013>2068>2085. However, volumetric soil moisture content was similar in 2068 and 2085 reflecting the prior rain input (Figure 1B). Similar to soil temperature, volumetric soil moisture content was also higher in S2 than in S1 in both climates. In S1, the relative soil humidity as determined by drying & weighting of soil subsamples at DAS131 and DAS159 was lower in planted soil than in unplanted soils. This was also the case for S2 in 2085, but in 2068 and 2013 planted S2 soil was wetter than unplanted S2 soil. The pattern was consistent at both DAS131 and DAS159 (SM1).

Table 1: Soil temperature and water content during the time of tea bag incubation (DAS 131-145). ‘Y’ and ‘NO’ indicate planted (YES) and unplanted (NO) soil respectively. See SM1 for soil moisture data separated for DAS131 and DAS159. *WR: winter room: no data from soil sensors.

	2013				2068				2085			
	S1		S2		S1		S2		S1		S2	
Air T □ C	2.15				7.91				10.72			
Soil T □ C	WR*		WR*		10.15		10.56		11.77		16.20	
Δ soil T □ C S2-S1	NA				+0.41				+4.43			
Cumulative precipitation mm m ⁻²	180.40				136.24				13.25			
Vol. soil moisture content (MC) %	WR*		WR*		34.19		38.47		34.45		37.99	
Δ soil vMC % S2-S1	NA				+4.27				+3.54			
Rel. soil MC %	Y	NO	Y	NO	Y	NO	Y	NO	Y	NO	Y	NO
Av. DAS131-159	18.91	21.65	21.92	10.34	17.84	20.72	19.77	17.85	12.81	16.74	15.35	19.64
Δ soil vMC % unplanted-planted	+2.74		-11.58		+2.88		-1.92		+3.93		+4.29	

155 2.4 Decomposition and further carbon and nitrogen cycling parameters

156 Within each of the studied cubes, n=5 rooibos and n=5 green tea bags were buried at 8cm
 157 depth to measure decomposition with the tea bag index from DAS131-145. The tea bag index
 158 integrates mass loss data from both green tea (fast-decomposing) and rooibos tea (slow-
 159 decomposing) into a single standardized k value combining information from the two
 160 substrates to represent the early-stage decomposition rate under asymptotic model
 161 assumptions (SM8)^[15]. The tea bag index is most indicative for litter decomposition, but tea
 162 is mineralized alongside soil organic matter and plant-derived compounds if present. In
 163 addition, in each cube interstitial soil pore water was extracted in situ using permanently
 164 installed soil pore water samplers (Rhizosphere Research Products B.V., Wageningen, The
 165 Netherlands) with the 10cm long porous membrane situated in the center of each cube
 166 vertically inserted at a depth of 8-15cm. Water was extracted weekly (DAS 82, 102, 110, 131,
 167 150) by applying a negative pressure using 20 ml syringes and metabolically relevant
 168 compounds were quantified immediately after water extraction using anthrone reaction for
 169 glucose equivalents (modified after [16]) and nitrate was quantified biochemically using an
 170 ion-selective electrode (LAQUAtwin NO3-11C, Horiba Ltd., Kyoto, Japan). At DAS 102,
 171 131 and 159, basal as well as exudate-induced respiration were quantified via MicroResp
 172 with two technical replicates for each modality^[17]. Soils were sieved to 2 mm and incubated
 173 within the CERs without further adjusting water content to maintain the soil samples as close
 174 to the humidity and temperature conditions of respective climate scenarios. Basal respiration
 175 was measured as CO₂ released from soil samples without any amendment, and as a proxy for
 176 exudate-induced respiration, 25µl of the collected interstitial soil pore water was used. At
 177 DAS131 and DAS159, microbial biomass and bacterial and fungal gene copies were
 178 measured in n=4 planted cubes per CER and the n=1 unplanted cube using direct chloroform
 179 extraction followed by titration (modified after [18]) and qPCR of 16S and 18S respectively
 180 (SM 2)^[19,20,21]. Sensors for continuous monitoring of soil humidity and temperature were
 181 installed in n=2 planted cubes per soil type per CER, but was not permanently monitored in
 182 unplanted cubes. Therefore, soil humidity was also quantified using the gravimetric method,
 183 drying sub-samples to constant mass, for all soils of planted and unplanted cubes which were
 184 also used for microbial biomass and qPCR at DAS131 and DAS159.

185

186 2.5 Statistical analysis

187 The individual and interactive effects of plant (YES,NO), climate (2013,2068,2085), soil type
 188 (S1,S2), and DAS (82,102,110,131,150) where applicable, on the response parameter
 189 decomposition, glucose, nitrate, microbial biomass, fungal and bacterial genes, and basal and
 190 exudate-induced respiration were individually evaluated with linear mixed models, with a
 191 random effect for cube ($y \sim \text{PLANT} * (\text{SOIL} + \text{YEAR} + \text{DAS}) + (1 | \text{CUBE})$).

192 To increase the resolution of the potential driving factors of decomposition (k) beyond the
 193 fixed effects climate (temperature, rain), soil (SOC, sand, humidity, temperature), plant (total
 194 root length, network area, branching frequency, number of root tips) and rhizosphere
 195 processes (glucose, nitrate, microbes), partial least squares regression (PLS) was
 196 performed^[22]. Leave-one-out cross-validation was used to assess model performance and the
 197 orthogonal scores algorithm was selected, as required for subsequent calculation of variable
 198 importance in projection (VIP) scores (SM3, SM4). Leave-one-out cross-validation (LOO-
 199 CV) was employed to assess model performance and determine the optimal number of

components. Missing values in the predictor matrix were imputed using median values per variable, and constant predictors were removed prior to scaling and centering the data. To complement the PLS analysis and capture potential non-linear relationships and interactions among predictors, a random forest (RF) regression model was fitted^[23]. The model used 500 trees and the default number of variables tried at each split for regression ($mtry = \text{floor}(p/3)$, where p is the number of predictors). The same imputed predictor matrix (without scaling) and response variable (k) were used as in the PLS model. Model performance was evaluated via out-of-bag (OOB) mean squared error and % variance explained (SM5, SM6). Both methods were applied to the same pre-processed dataset to enable direct comparison of predictor importance rankings. Overlap in the top-ranked variables across PLS ($VIP > 1$) and random forest (%IncMSE) provides robust support for the key drivers of decomposition.

Data was analyzed using statistical software R^[24], with the additional packages `car`^[25], `caret`^[26], `dplyr`^[27], `effects`^[28], `emmeans`^[29], `ggnewscale`^[30], `ggplot2`^[31], `lme4`^[32], `lmerTest`^[33], `patchwork`^[34], `performance`^[35], `pls`^[36], `plsVarSel`^[37], `randomForest`^[38], `tibble`^[39], `tidyr`^[40], and `tidyverse`^[41].

1. Results

3.1 Decomposition constant k

Plant presence significantly influenced the decomposition constant k ($F_{1,1} = 10.64$, $p = 0.031$), with higher values in unplanted (NO) compared to planted (YES) cubes when averaged across soil types and climate scenarios (Figure 2). A marginal main effect of climate scenario was also observed ($F_{1,1} = 6.27$, $p = 0.058$), whereas soil type had no significant effect ($F_{1,1} = 0.21$, $p = 0.674$). No significant interactions were detected between plant presence and soil type (PLANT $\times$ SOIL: $F_{1,1} = 3.76$, $p = 0.125$) or between plant presence and year (PLANT $\times$ YEAR: $F_{1,1} = 4.48$, $p = 0.095$), indicating that the negative effect of plant presence on decomposition rate was relatively consistent across soil types and climate conditions. However, there was a tendency for higher decomposition in S2 than in S1 in unplanted soils, most notably in 2013, and higher decomposition in S1 than in S2 in presence of plant, most notably in 2085.

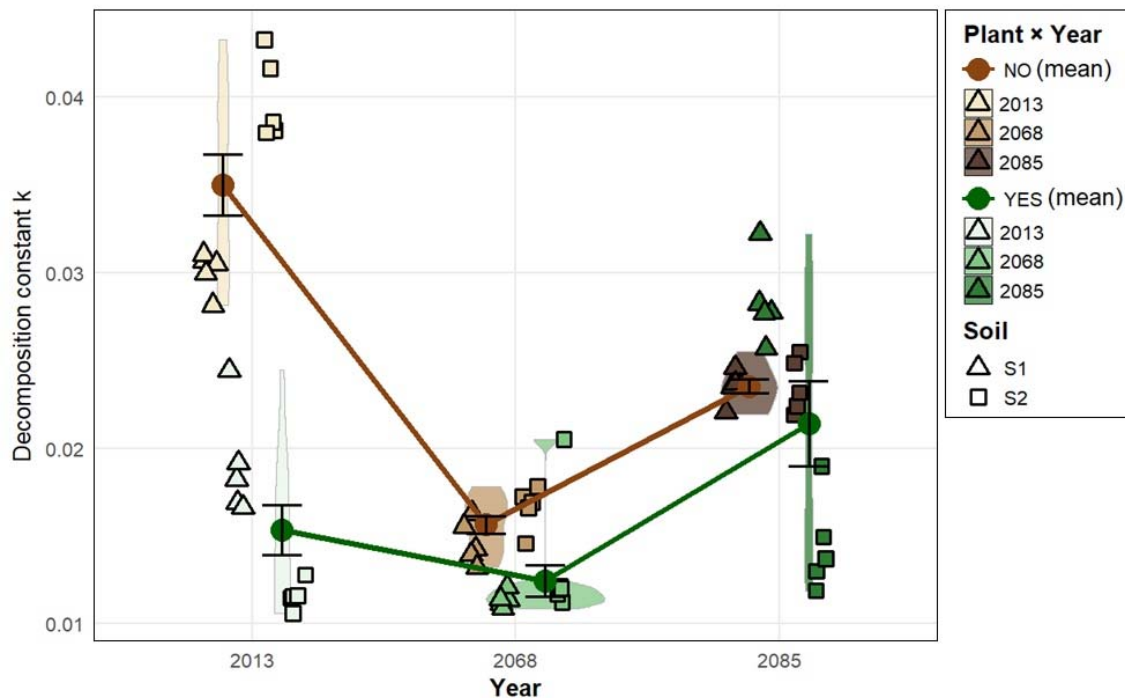


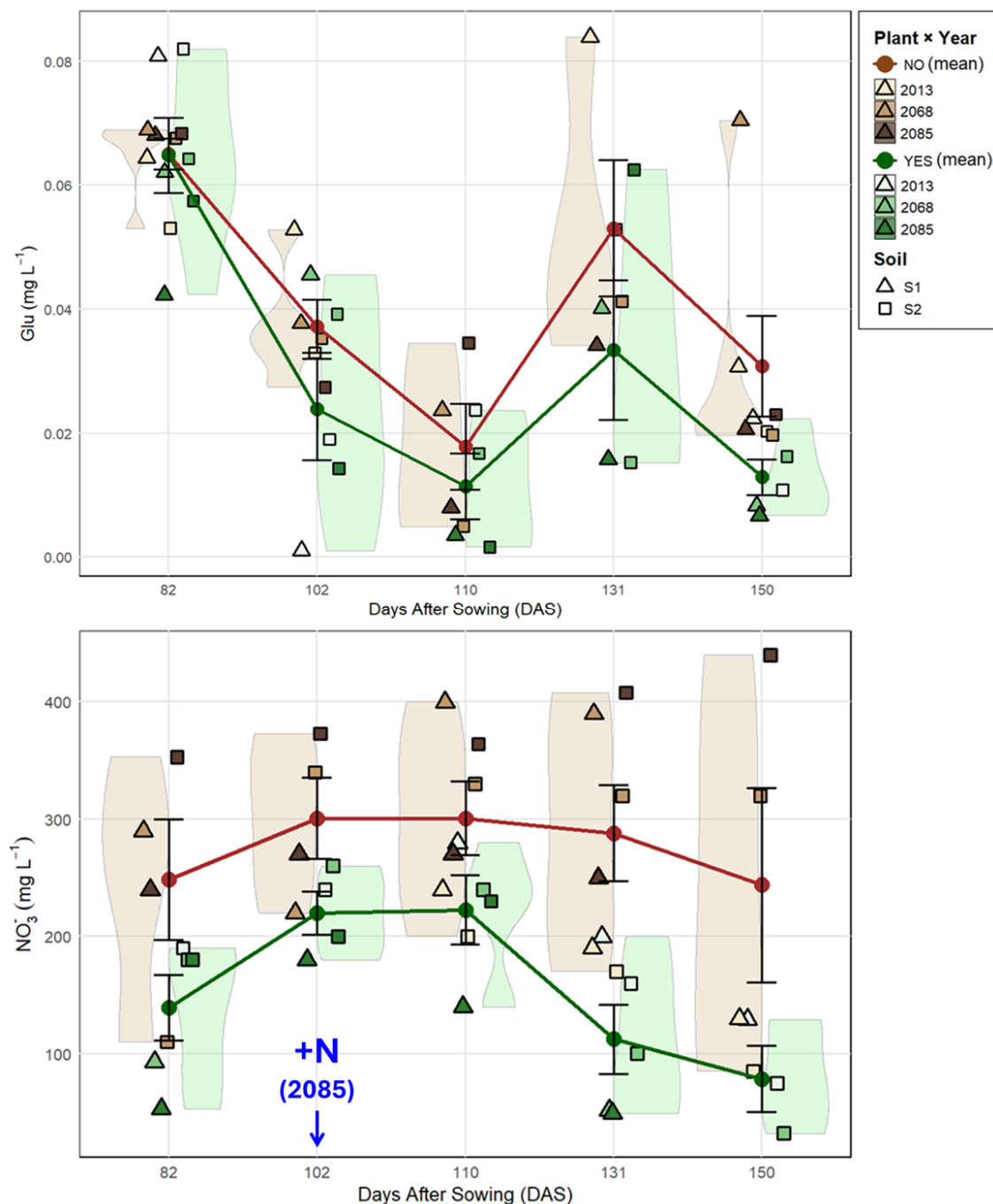
Figure 2: Decomposition constant k as determined by the tea bag index in planted (YES) and unplanted (NO) soils in three climates (2013, 2068, 2085) under two soil management histories (S1: low OM, S2: high OM). Triangles and squares represent the technical replicates (individual tea bags) within each soil x climate modality ($n=5$) and circles and lines represent respective means.

3.2 Glucose-equivalents and nitrate in interstitial soil pore water

For glucose levels in interstitial soil pore water, linear mixed-effects models revealed a significant interaction between plant presence and days after sowing (PLANT $\times$ DAS: $F_{1,11} = 13.69$, $p < 0.001$), where planted cubes exhibited lower soil glucose concentrations than unplanted controls at most time points (Figure 3 upper panel). No significant interactions were found between plant presence and soil type (PLANT $\times$ SOIL: $F_{1,11} = 1.46$, $p = 0.293$) or climate scenario (PLANT $\times$ YEAR: $F_{2,22} = 0.049$, $p = 0.953$), suggesting that the plant-induced reduction in glucose was consistent across soil types and climate conditions. There was an overall decline of glucose concentration in interstitial soil pore water by -0.0045 units per 10 days from DAS82 to DAS150, with a spike at DAS131. When averaged across all sampling dates, the main effect of plant presence was not significant ($p = 0.110$), and neither soil type ($p = 0.720$) nor year ($p = 0.439$) had significant main effects.

Plant presence also had a significant overall effect on nitrate levels which were lower in planted than in unplanted cubes ($F_{1,11} = 9.70$, $p = 0.036$), most likely indicating plant N-uptake (Figure 3 lower panel). This effect was modulated by sampling time, as indicated by a significant PLANT $\times$ DAS interaction ($F_{1,11} = 98.14$, $p < 0.001$). A marginal interaction between plant presence and climate scenario was also observed (PLANT $\times$ YEAR: $F_{2,22} = 6.16$, $p = 0.060$), whereas no significant interaction with soil type was detected (PLANT $\times$

254 SOIL: $F_{\square, \square} = 0.063$, $p = 0.814$). Neither soil type ($p = 0.496$) nor year ($p = 0.565$) exerted

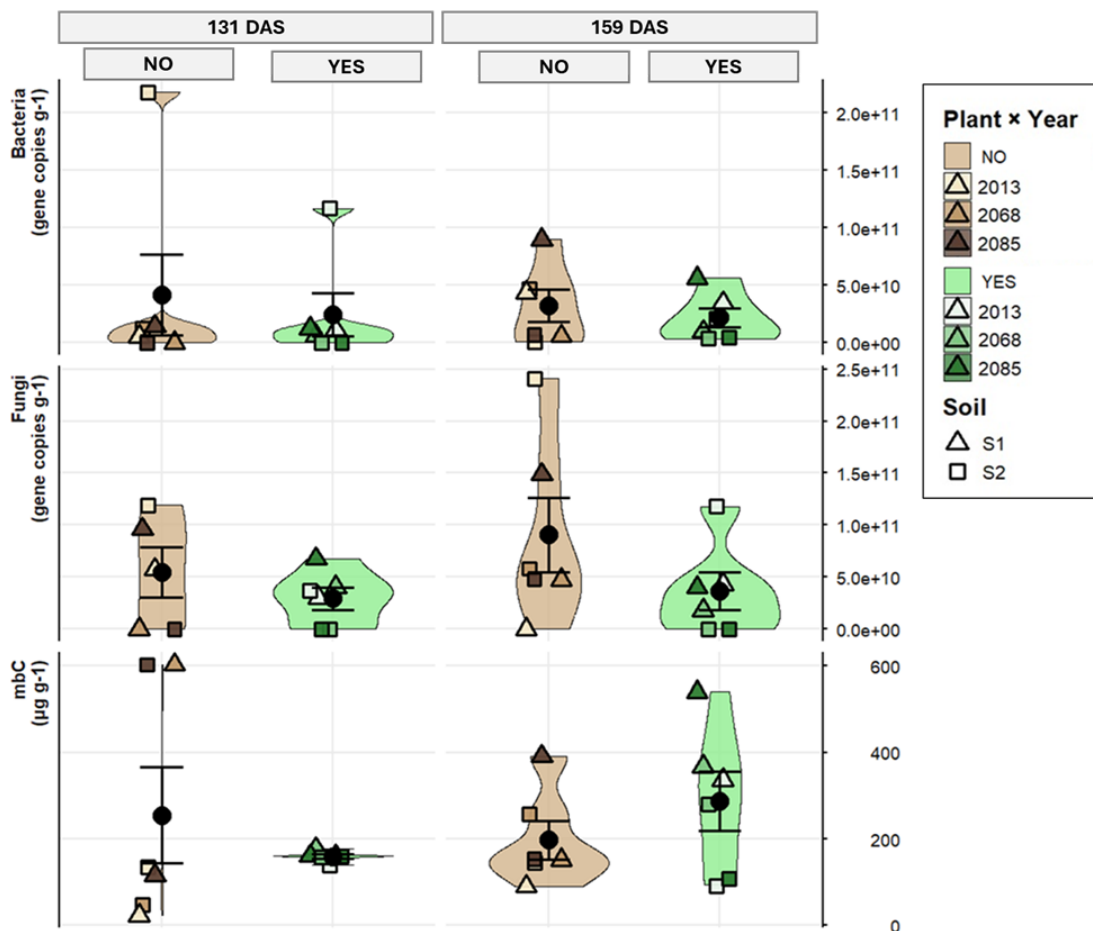


255 significant main effects.

256 Figure 3: Glucose (upper panel) and nitrate (lower panel) dynamics in interstitial soil pore
257 water determined at five days (days after sowing (DAS)). Triangles and squares represent one
258 value per soil x climate modality, totaling between four and six values for each level of the
259 main factor 'plant' (planted=YES and unplanted=NO) at each day, depending on the
260 extractability of interstitial soil pore water. Note: Mineral nitrogen fertilizer (NO₃NH₄) was
261 applied at DAS102, only in 2085 and only in planted cubes.

3.4 Microbial biomass carbon, bacteria and fungi

No significant main effect of plant presence on bacterial gene copy numbers, fungal gene copy numbers or microbial biomass carbon (mbC) was detected at 131DAS and 159DAS (all $p > 0.43$). Time (DAS) also had no significant effect on bacterial gene copies ($F_{1,12} = 0.071$, $p = 0.796$), fungal gene copies ($F_{1,12} = 2.23$, $p = 0.170$) and microbial biomass C ($F_{1,12} = 0.239$, $p = 0.635$). Similarly, no significant interactions were detected in any variable and neither soil type nor year showed statistically significant effects. However, on average the measured parameter were higher in unplanted than in planted soils, apart from



mbC at 159DAS.

Figure 4: Bacteria (16S gene copies g soil⁻¹) and fungi (18S gene copies g soil⁻¹) abundance and microbial biomass carbon (mbC g soil⁻¹) at two time points (DAS131, DAS159) in unplanted (NO) planted (YES) cubes in two soil types (S1: low OM, S2: high OM) in three climates (2013, 2068, 2085). Triangles and squares represent one value per soil x climate modality, totaling six values for each level of the main factor 'plant' (planted=YES and unplanted=NO) at each day.

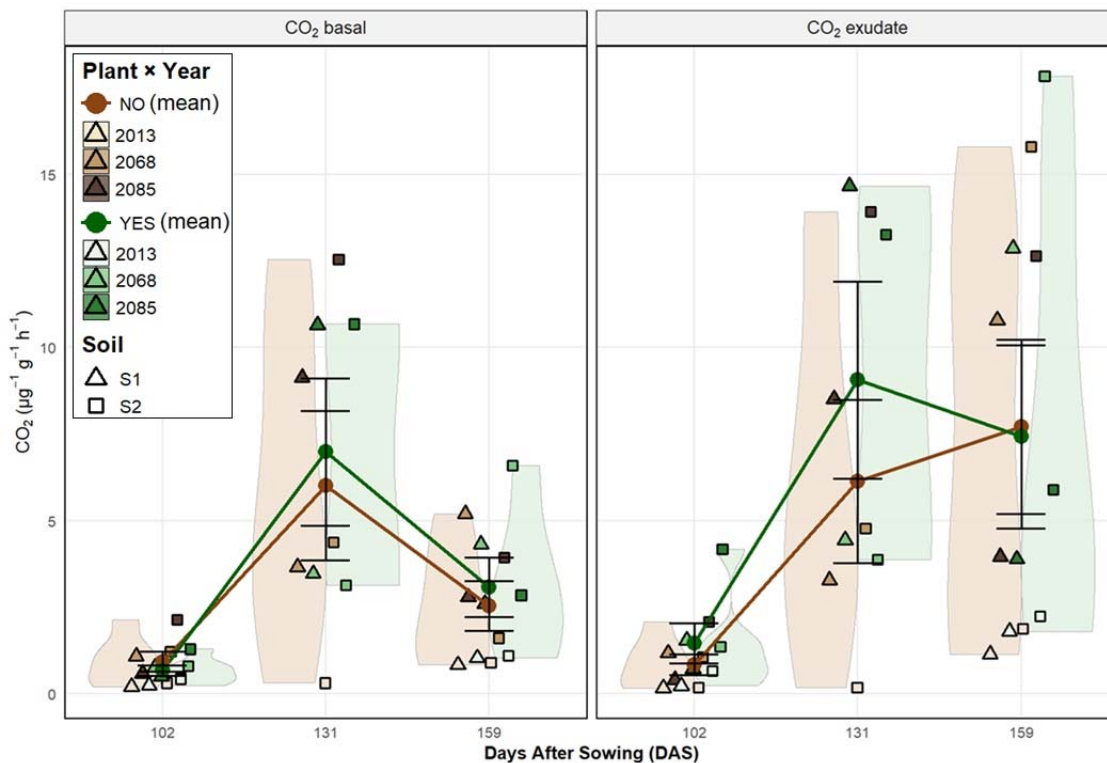
3.5 Basal and exudate-induced soil respiration

Repeated-measures linear mixed models with random intercept for "cube" were fitted separately to basal and exudate-induced CO₂ data. There was no overall significant effect of

280 plant on either CO₂ parameter, but both CO₂ parameters were higher in the future climates
281 than in the past climate.

282 Specifically, basal CO₂ showed no significant influence of plant presence (main effect:
283 $F_{1,12} = 0.168$, $p = 0.686$) and no significant interaction with time (PLANT $\times$ DAS:
284 $F_{2,24} = 0.133$, $p = 0.877$). Significant effects were observed for DAS ($F_{2,24} = 10.95$, $p =$
285 0.00055) and year ($F_{2,24} = 4.80$, $p = 0.019$) but not soil type ($p = 0.802$).

286 Exudate-induced CO₂ likewise showed no significant main effect of plant ($F_{1,12} = 0.317$,
287 $p = 0.580$) or PLANT $\times$ DAS interaction ($F_{2,24} = 0.216$, $p = 0.807$). Time (DAS: $F_{2,24} =$
288 6.80 , $p = 0.0053$) and year ($F_{2,24} = 4.43$, $p = 0.025$) were significant, whereas soil type
289 had no effect ($p = 0.245$).



290
291 Figure 5: Basal (left) and exudate-induced (right) soil respiration (µg CO₂ g soil⁻¹ hour⁻¹) in
292 planted and unplanted cubes in two soil types (S1: low OM, S2: high OM) in three climates
293 (2013, 2068, 2085) at three timepoints (DAS102, DAS131, DAS159). Circles and triangles
294 represent one value per modality, each of which is the mean of two technical replicates. This
295 totals six values for each level of the main factor 'plant' (planted=YES and unplanted=NO) at
296 each day.

297 3.6 Integrative analysis

298 Despite several climate, soil and root parameter being included, the most powerful predictors
299 of decomposition were microbial and metabolic factors, above all fungal gene copies and
300 microbial respiration (CO₂) which was constituent for both RF and PLS models (Figure 6).
301 The most significant environmental variable was average soil moisture content (Av.soil.MC).
302 Strong agreement between the two independent methods supports notably the importance of
303 fungal gene copies at 131 DAS, alongside consistent high rankings for nitrate (NO₃⁻)
304 concentrations, CO₂ respiration (basal and exudate-induced), microbial biomass carbon

(mbC), and related rhizosphere indicators at later sampling points. Partial dependence plots (SM7) revealed strong positive associations between the decomposition constant k and fungal gene copies at 131DAS, nitrate concentrations at 131DAS, microbial biomass carbon at 159DAS and glucose at 150DAS, indicating these as key drivers of decomposition. In contrast, negative associations were observed for exudate-induced CO_2 respiration at 102DAS and basal CO_2 respiration at 159DAS (SM7). Nonetheless, the proportion of variance explained by RF was substantially higher than by PLS (95% vs 18%) indicating that interactions among the included variables are also important.

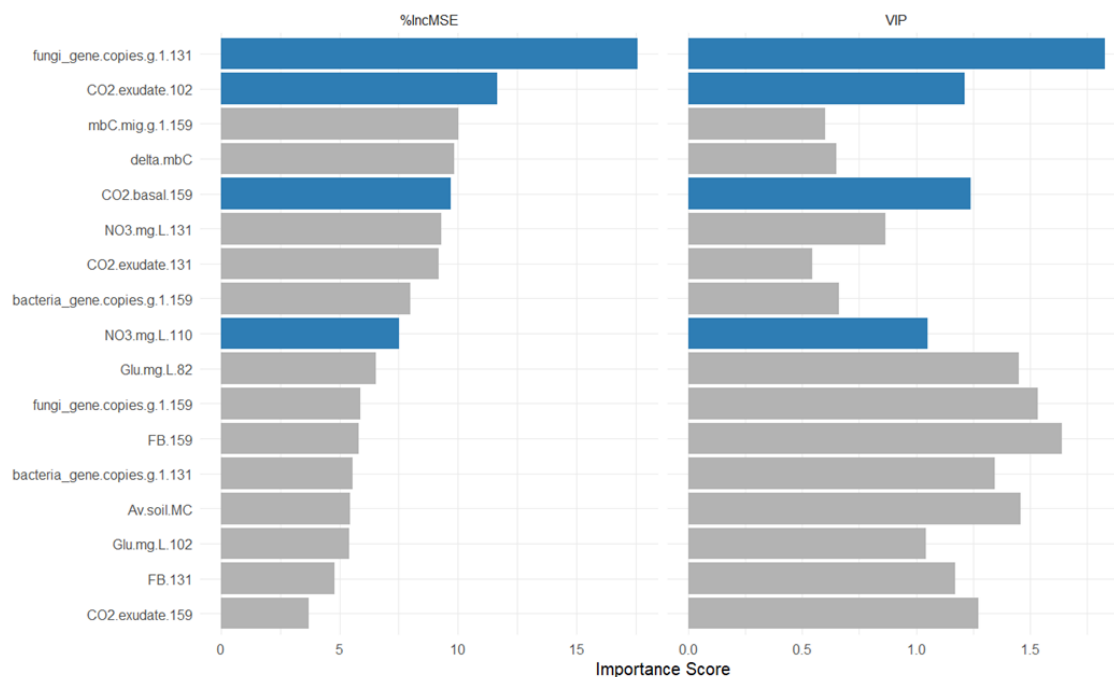


Figure 6: Variable importance comparison for predicting the decomposition constant k using Random Forest (RF) regression (left) and Partial Least Squares (PLS) regression (right). Bars represent importance scores for predictors meeting at least one of the following criteria: Variable importance in projection (VIP in PLS) > 1 or percentage increase in out-of-bag mean squared error when the variable is randomly permuted (%IncMSE in RF) > 7 (breakpoint cf SM6). Variables which meet both criteria are highlighted in blue.

4. Discussion

4.1 Plant-microbe competition (hypotheses under premise 1)

Premise P1 posited that plant exudates of carbohydrate-rich substances enhance microbial mineralization^[1,2,3,4]. Contrary to this, results indicate that plant presence slowed rather than amplified decomposition (Figure 2), which has been observed previously for grasses and forbs^[42]. The lower decomposition in planted than in unplanted cubes was in line with lower glucose and nitrate concentrations in planted cubes, leading to rejection of HP1a and HP1b. Under the given measurement conditions and time frame, no overall differences emerged in soil-emitted CO_2 , microbial biomass, or bacterial/fungal gene copies, leading to rejection of HP1c and HP1d. These results align with resource competition theory between plants and microbes, which posits that plants can outcompete microbes over nutrient acquisition, inducing N-limitation for microbes and thus delaying decomposition^[5,7,43,44,45]. Every

molecule of nitrogen which goes into belowground biomass of microbes or fauna, is a molecule of nitrogen which is not immediately available for plant uptake. Microbes thus often dominate short-term N-competition, but over longer timeframes competition for nitrogen is often asymmetrical in favor of the plants, sometimes accompanied by positive rhizosphere priming effects that enhance microbial SOM-mineralization^[1,46,47,48]. This asymmetry is context-dependent, but ¹⁵N-labeling studies indicate that plants ultimately acquire more fertilizer-derived N than microbes, particularly in the rhizosphere^[6,49]. Implementing such dynamics into concepts of "soil health" is important, as high microbial abundance and biomass can present a significant N-sink and thus antagonize plant growth^[5,10,50]. Whether or not belowground organisms benefit plant growth depends on food-web structure, on the type of relationship established between symbiotic organisms and plants (mutualism, commensalism, parasitism), and whether catabolism or anabolism prevails in soil organisms^[51,52]. To optimize interactions between crops and soil organisms for improved plant production, future research could identify parameters which allow to distinguish facilitative and antagonistic organisms, including links to carbon and nutrient exchange dynamics^[4,53,54,55,56].

4.2 Nitrate homeostasis (hypotheses under premise 2)

Under premise P2 we assumed that plants take up nitrogen and that adding mineral nitrogen fertilizer compensates or even exceeds plant N-uptake and thence expected to observe an increase in nitrate concentration in interstitial soil pore water in 2085 (or a less steep decrease). The addition of fertilizer at DAS102 in 2085 did not result in measurable differences in interstitial soil pore water, which increased also in the unfertilized planted soils and in the unplanted soils at that moment of measurement, leading to rejection of H2a (Figure 3). While we observed lower nitrate concentrations in planted than in unplanted soils, we reject H2b at $\alpha=0.05$, despite robust indication for substantial plant N-uptake (average depletion -91 mg L^{-1} , $p = 0.08$).

Similarly, there was no indication that warmer temperatures in future climate scenarios stimulated microbial activity during the study period, accelerating decomposition and thus enhancing interstitial soil pore water. While this observation is in line with other studies^[57,58], the absence of a detectable effect of fertilizer addition or temperature on soil NO_3^- does not constitute evidence of absence of such effects, as this result might have been caused by the measurement conditions, where rapid plant and microbial N-uptake could have obscured the performed measurements in interstitial soil-pore water. Plants exhibit high-affinity uptake systems for NO_3^- , allowing quick absorption from soil solution especially during periods of active growth like the tillering stage in cereals^[59]. Over time, soil nitrogen concentrations could also be stabilized as a function of the net balance of nutrient inputs and outtakes in the belowground multi-trophic networks, also known as the 'microbial loop,' wherein the predation of bacteria and fungi by microbial grazers, such as protozoa and nematodes, triggers the mineral release of previously immobilized nitrogen back into the rhizosphere^[60,61].

In addition, variable precipitation, especially reductions or erratic patterns, can compound nutrient losses by drought-stress induced disruptions of microbial community structures and reduces enzyme activities involved in nutrient retention, potentially leading to higher losses of both carbon and nitrogen^[58,62,63]. In such scenarios, soils with diverse but less resilient

microbial communities, for example those dominated by fast-growing opportunists, may experience amplified nutrient volatilization or erosion, particularly in agricultural or grassland systems^[64,65].

4.3 Role of stoichiometry

Reduced decomposition has been previously observed for various grasses and forbs and it has been proposed that this is driven by elevated C:N ratios of root exudates, providing microbes with abundant energy but insufficient nitrogen^[42,66]. This stoichiometric imbalance can trigger microbial nitrogen sequestration (immobilization), when microbes prioritize the uptake of scarce inorganic N like nitrate and ammonium to support their own biomass growth^[6]. When the substrate to be decomposed is also nutrient-poor with high C:N ratios, which is the case for organic matter from plant residues like straw and tea bag litter, it can result in enhanced competition for nitrogen between plants and microbes leading to a net decrease in decomposition rates due to severe nutrient limitation within the microbial community. The typical C:N ratio of wheat straw ranges from 80:1 to 100:1, though it can occasionally be as high as 120:1 depending on the variety and fertilization history^[67]. Because these values vastly exceed the critical C:N threshold of approximately 25:1 to 30:1, microbial demand for nitrogen surpasses the supply available in the residue, leading to net N sequestration (immobilization) in the microbial biomass rather than mineral release^[68]. Green Tea has a C:N of approximately 10:1 to 12:1, which is below the critical threshold (< 25:1). Thus, it undergoes faster mineralization than rooibos tea, which has a C:N of approximately 40:1 to 60:1 and is thus more prone to trigger nitrogen competition (SM8)^[15,69].

4.4 Fungi and humidity are more important for decomposition than bacteria and temperature

In multivariate analysis including air and soil temperature and precipitation and soil moisture, as well as several soil and root parameters, microbial and metabolite parameters remained the strongest in predicting decomposition, in particular fungal gene copies and CO₂ as an indicator of microbial activity. Fungi are generally associated with the breakdown of more complex molecules, while bacteria are thought to feed primarily on LMW sugars, which is in line with this result^[70,71]. However, these roles are highly context-dependent. For example, some rhizosphere fungi can outcompete bacteria for labile exudates under nutrient-limiting conditions, and selective grazing from protists and nematodes can also shift fungal-to-bacterial ratios thereby altering decomposition and N-availability^[72,73]. However, there was no interaction effect with nitrogen addition in 2085, which has previously been shown to affect wheat straw decomposition through altered bacteria and fungi dynamics^[74]. Soil moisture was the only environmental parameter having a considerable influence on the model's predictive power (Figure 6). Soil moisture influences decomposition and microbial activity by regulating oxygen availability and substrate diffusion^[75,76], which possibly contributed to the increased decomposition rates particularly in unplanted cubes in 2013, which was the wettest year during the study period (Figure 1). Decomposition is generally considered to be also temperature sensitive^[77]. Temperature's lack of relevance in this multivariate context could have stemmed from its effects being constrained or masked by other factors such as moisture or microbe-substrate interactions^[78]. On the other hand, previous studies have found that microbial decomposition can be very resistant to

temperature^[79,80], so the absence of temperature effects on decomposition could also indicate true absence of ecological relevance in this context.

4.5 Possible impact of winter conditions on soil processes in 2013

The biggest difference in decomposition between planted and unplanted cubes was observed in the 2013 climate, which was also the year where plants and soils experienced winter conditions with below zero temperature (Figure 1, Figure 2). Freeze-thaw cycles can disrupt microbial cells and soil aggregates, leading to cell lysis and the release of organic matter, including labile carbon and nitrogen compounds^[81]. This "thaw burst" can enhance microbial mineralization rates as surviving or recolonizing microbes rapidly decompose the newly available substrates, increasing nutrient cycling and CO₂ emissions in the short term^[82]. In the unplanted soils, all elements released through this process were available to microbes, increasing decomposition. In the planted soils, plants also took up some of the nutrients released by microbial turnover, thus lowering resources for microbes and therewith their metabolic activity in mineralizing organic materials. Prolonged freezing can also reduce overall microbial biomass and diversity over winter, delaying mineralization recovery^[83]. If thaw coincides with erratic precipitation, it can lead to nutrient losses via leaching and/or denitrification, reducing net mineralization efficiency, which aligns with the lower overall NO₃⁻ and respiration in 2013 (Figure 3, Figure 5). Microbes often outcompete plants for N post-thaw due to higher surface area and rapid growth, leading to temporary N-immobilization and suppressed plant uptake^[84]. This could explain the lower decomposition, and NO₃⁻ in planted soils in 2013 as plants can exacerbate resource scarcity. In unplanted soils, microbial activity is not limited by resource drain from plant nutrient uptake, possibly amplifying differences between planted and unplanted soils. Warmer winters in the future could reduce the nutrient flush from thawing events, leading to more balanced nutrient pools but potentially higher overall microbial N-sequestration if their activity is not limited by carbon or other factors.

4.6 (No) differences between soil types

In the low organic matter soil S1, the unplanted soil was wetter than the planted, most likely due to plant water-uptake in the planted cubes^[85]. Interestingly, in the high organic matter soil S2, the unplanted soil was drier than the planted soil in 2013 and 2068, possibly due to increased drainage and/or evapotranspiration in unplanted S2 cubes, and/or due to increased water-holding capacity of planted cubes in S2 in these years^[86,87]. Decomposition rates were higher in unplanted S2 in 2013 and in planted S1 soils in 2085 (Figure 2). This aligns with resource competition dynamics, where plant presence may suppress decomposition in high-SOM soils via nutrient limitations on microbes, while enhancing it in low-SOM contexts through priming effects, though this was not reflected in soil CO₂-emissions as measured here^[42,46]. Notably, this mirrors plant performance and yield trends, which were higher in S1 than S2, likely because lower SOM in S1 reduces microbial competition for nutrients, allowing greater crop access and productivity, while higher SOM in S2 supports faster microbial decomposition but may limit yields through antagonistic interactions or nutrient immobilization, in contrast to expectations^[12,10,50,88].

Despite these contrasting soil water dynamics between S1 and S2, measured parameters like decomposition, respiration, and glucose equivalents showed minimal differences overall, indicating that this process is either driven by stoichiometry as both soils have a C:N ratio of

10.5, and/or that plants and soil microbes can buffer against water and temperature variability to sustain homeostasis in nutrient cycling and microbial activity^[89,90]. This resilience aligns with findings of no significant grain quality differences between these two soil types in the same experiment^[91], though climate exerted a broad negative impact on grain quality, highlighting the sensitivity of crops to abiotic stressors despite biotic buffering.

4.7 Implications for agroecosystems under climate change

The slowing down of decomposition in the presence of growing plants as demonstrated here and elsewhere^[42] supports the integration of cover crops in rotations as they can prevent N-leaching and provide habitat for diverse species^[92]. It has also been suggested that inhibition of decomposition by ecto-mycorrhizal fungi in forested ecosystems resulting from plant-microbe-competition can increase soil C storage^[93,94], which could also be a mechanistic explanation for increased soil C-stocks described under cover crops^[95,96]. However, the results of this study also indicate that soil management and fertilization practices can still be improved to synchronize soil nutrient provision with plant nutrient demand, particularly at critical growth stages like stem elongation. One possible lever to improve N-availability for plants which might become even more relevant under future climates is nitrification inhibition, either synthetic or biological^[97,98]. Another path towards mitigating the risk of nutrient losses to plant-microbe competition could be increasing the inherent capacity of cereals to acquire nitrogen-fixing microbes^[99]. Similarly, practices like intercropping or integrating legumes into crop rotations could help balance plant-microbe dynamics and promote steady mineralization while mitigating nutrient losses to competition, however this does not always benefit yields^[100,101]. The results of this study also reinforce the benefits of split fertilization in cereal systems, as nutrient demands at critical growth stages can be high necessitating readily plant-available nitrogen to support plant growth and fitness, and ultimately yields and protein contents^[102].

4.8 Limitations & outlook

The present study covered a period of 14 days for the assessment of decomposition via the tea bag index, which is an appropriate timeframe to capture the majority of overall mass loss from tea bags and differences between green and rooibos tea (SM8)^[15]. Yet we acknowledge that this period represents solely the conditions around the stem elongation growth stage of the wheat plants in the respective climates. Decomposition dynamics may be different at different growth stages^[47,103] which were not studied in the present experiment to avoid confounding factors such as diverting growth stages due to staggered development and differences in external inputs from multiple doses of fertilization (Table 1, Figure 2). Further, given that microbial processes and plant N-uptake can be fast (seconds-hours), it is possible that some of the dynamics were missed despite the methods used being very sensitive. Finally, the main factor of this experiment was plant presence or absence, for each of which we had six independent replicates, but nested within the main factor 'plant' were the factors soil and climate for which only technical replicates exist. While we can have confidence in the main plant-effect which was suppression of decomposition consistent across all soil x climate modalities and in line with previous studies^[42], future experiments could increase the replication for nested factors like soil type and climate to further nuance the findings. Future studies could also increase the spatial and temporal resolution of data acquisition e.g. by

506 increasing rates of sampling for highly dynamic parameter, installing continuous nitrate
507 sensors and potentially tracking C and N molecules using stable isotopes.

508 **5. Conclusion**

509 Overall, this study supports the inclusion of plant-microbe competition and of the sink
510 strength of belowground networks into nutrient cycling models for the rhizosphere. By
511 prioritizing approaches that account for these interactions, crop production can be tailored
512 towards reliable soil nutrient provision and adapted to altered plant nutrient demand under
513 varying meteorological conditions. Future research with broader temporal scales could
514 further define these insights for different plant growth stages. Quantifying the sink strength of
515 belowground trophic networks could also inform the timing of fertilizer use to reduce the risk
516 of nutrient losses especially when plant demand is high. Future management strategies could
517 leverage the observed plant-microbe dynamics to enhance the ability of the microbial loop to
518 stabilize nitrogen against leaching during extreme weather while simultaneously improving
519 the synchrony between mineralization and the spatio-temporal nutrient demands of crop
520 plants. By optimizing this biological buffer system, agroecosystems can achieve greater
521 climate resilience, maintaining both soil health and productivity under increasingly volatile
522 environmental conditions.

523 **Data availability**

524 All data presented in this manuscript is available at
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