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1 **Tensions in tillage: Reduction in tillage intensity associates with lower wheat growth**
2 **and nutritional grain quality despite enhanced soil biological indicators**

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26 ## Abstract

27 Dryland ecosystems are particularly susceptible to the adverse effects of intensive agriculture,
28 with intensive tillage exerting a major impact on soil health and its biotic components. The
29 implementation of less disturbing soil management practices can be essential for preserving
30 the soil environment and maintaining the diverse communities of microorganisms, micro- and
31 mesofauna, which are essential contributors to soil fertility. In this study, we assessed soil
32 chemical properties, soil biodiversity and functionality, and wheat crop growth across a tillage
33 gradient encompassing no-tillage (NT), minimum tillage (MT), and standard tillage (ST).
34 Results showed that NT resulted in increased soil macronutrient levels compared to MT and
35 ST. In general, reduced tillage increased the abundance of soil biota, with significantly higher
36 levels of bacterial and fungal marker genes observed in MT and NT compared to ST.
37 Nematode abundance increased by 25% in MT and 50% in NT, compared to ST and predatory
38 acari were significantly more abundant in NT, while numbers of total acari were higher in both
39 NT and ST compared to MT. Community structure analysis revealed that tillage strongly
40 influenced bacterial, fungal and acari community composition, reflecting a gradient of soil
41 disturbance intensity. Corresponding to the increased abundance of soil biota, reduced tillage
42 increased microbial activity and soil functionality along the disturbance gradient. This was
43 evident in the potential activity of carbon, nitrogen and phosphorus cycling enzymes, as well
44 as the microbial capacity for carbon utilisation. In addition, evidence of the formation of biocrust
45 as a possible source of carbon input was found. Furthermore, we observed important wheat
46 pathogens to decrease and fungal antagonists to increase in NT compared to ST. Despite
47 enhanced soil biological indicators under reduced tillage, wheat growth, nitrogen uptake and
48 grain B vitamin contents were higher in ST compared to NT. In addition, we observed a shift
49 in technological grain properties across tillage practices. The higher root:shoot ratio (an
50 indicator of nitrogen deficiency) and median root diameter (hormone-driven lateral expansion)
51 in NT suggest that soil compaction could be a potential cause of reduced wheat performance.
52 These results suggest that despite improved soil biological indicators, other factors such as a

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53 low rates of N mineralization potential and prevalence of soil compaction may be limiting wheat
54 performance in NT systems.

55 **Keywords**: soil biodiversity; soil functioning; root structure; tillage practices; winter wheat;
56 grain B vitamins, grain technological properties

57 Highlights

58 • Enhanced microbial activity and functionality under reduced tillage
59 • Tillage intensity shaped community structure of microbes, nematodes and acari
60 • Soil biocrust development under NT may increase soil organic carbon
61 • Root traits revealed soil compaction and nutrient limitation in NT systems
62 • Reduced tillage impaired wheat quality and changed technological grain properties

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63 ## 1. Introduction

64 Dryland agro-ecosystems are particularly vulnerable to the detrimental effects of intensive
65 tillage, which causes upturn and disruption of soil structure, reduction of soil organic matter
66 (SOM) content, and ultimately altering soil biodiversity and functioning (Degrune et al., 2016;
67 Haddaway et al., 2017; Kraut-Cohen et al., 2020; Li et al., 2019; Peng et al., 2020). As studies
68 have shown, reducing tillage intensity has the potential to increase topsoil SOM, reduce soil
69 erosion, enhance water retention, and thus it can help to preserve the integrity of soils
70 (Haddaway et al., 2017; Holland, 2004; Lal, 2009).

71 Soil organisms provide multiple agroecosystem services including nutrient cycling, mitigation
72 of biotic and abiotic stresses and thus soil and plant health (Bender et al., 2023; Delgado-
73 Baquerizo et al., 2016, Delgado-Baquerizo et al. 2020; Wagg et al. 2019, Wagg et al., 2021).
74 Several studies have documented the beneficial impacts of reduced tillage on increasing the
75 abundance of soil microorganisms (Chen et al., 2020; Essel et al., 2019; Helgason et al., 2009;
76 Mathew et al., 2012; Morugán-Coronado et al., 2022) and micro- and mesofauna (Brévault et
77 al., 2007, Postma-Blaauw et al. 2010, Betancur-Corredor et al. 2022).

78 Through the disruption of fungal mycelia, intensive tillage is suspected to negatively impact
79 fungal communities (Roger-Estrade et al. 2010), while higher soil organic carbon (SOC) under
80 reduced tillage practices should enhance microbial abundance and activity (Lori et al. 2017,
81 Ramírez et al., 2020). However, a recent meta-analysis found that the response of soil
82 microbial diversity to tillage is highly variable and strongly depends on pedoclimate and plant
83 growth stage (Li et al., 2024, de Graaff et al., 2019): While minimum tillage increased bacterial
84 diversity by 7%, there was no significant effect on fungal diversity compared to ploughed
85 systems. Similarly, Capelle et al. (2012) have shown that the effect of tillage intensity on the
86 abundance of soil biota might differ depending on soil texture. Given the central role of local
87 pedoclimatic conditions in modulating the impact of tillage practices on soil microbial
88 communities, it is essential to approach each environmental context individually to gain a full
89 understanding of their responses. This involves to further identify key organisms responsible

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90 in shaping the soil environment, including those within the soil micro- and mesofauna, which
91 are often neglected.

92 So far, most field studies investigating belowground effects of tillage practices have focussed
93 either on the abundance and/or community structure of soil biota or on soil functional properties
94 but without a link to aboveground performance (Capelle et al. 2012, Roger-Estrade et al. 2010).
95 Furthermore, there is still limited understanding about the driving factors behind crop
96 performance under different tillage practices, as literature presents contradictory results: the
97 effects of no-tillage and minimum tillage reported in meta-analyses are ranging from negative
98 to neutral effects (Young et al. 2021). The meta-analysis of Pittelkow et al. (2015)
99 demonstrated best performance of no-tillage under rainfed conditions in dry climates, matching
100 conventional tillage yields, while average yield of 50 crops reduced by 5.1%. Thus, further
101 studies are needed that link soil biodiversity and functioning with crop growth and grain quality.

102 In the present study we investigated how tillage intensity in a long-term field experiment affects
103 soil properties and the interplay between soil biodiversity, functionality and crop performance.
104 Winter wheat was grown in crop rotation comparing no-tillage (NT), minimum/reduced tillage
105 (MT) and standard inversion tillage (ST). We assessed soil chemical, biological and functional
106 properties to identify tillage practices enhancing belowground functioning. Soil chemical factors
107 comprised a range of soil macro and micronutrients and soil pH. Soil biological variables
108 included the abundance, diversity and community structure of soil micro- and macrobiota
109 including bacteria, fungi, nematodes and acari. Soil functional properties focused on the C-, N-
110 and P-cycling potential of the soil, substrate utilization potential and microbial activity in
111 general. Further, in an effort to establish connections to various agronomic qualitity indicators
112 of winter wheat, we characterised shoot and root traits, grain nutrients and B vitamins as
113 indicators for nutritional quality as well as starch size distribution and gliadin and glutenin
114 contents in grains as technological quality indicators.

115 We hypothesised that: i) tillage intensity would be negatively correlated to SOC, soil
116 biodiversity and functionality, ii) tillage intensity would drive shifts in the community structure

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117 of soil biota, namely bacteria, fungi, nematodes and acari, and iii) that an increase in SOC and
118 higher nutrient cycling potential could translate into an enhanced uptake of nutrients leading
119 to higher wheat biomass and grain nutrient and B vitamin content.

120 2. Material and Methods

121 2.1 Site and soil characteristics

122 The experimental field for this investigation was the "La Canaleja" long-term tillage comparison
123 trial of the Spanish National Research Institute for Food Research and Technology (INIA-
124 CSIC), established in 1994 outside Alcalá de Henares (Madrid, Spain, 40°30'55.0" N
125 3°18'37.1" W; 600 m a.s.l.). The soil texture is classed as a Loam (USDA classification) and
126 the soil type as a calcic Haploxeralf. The climate is semi-arid Mediterranean with the majority
127 of the average annual precipitation of 367 mm yr^{-1} [1994-2022] having occurred in the autumn,
128 winter, and spring months. In recent years, the average monthly temperature in summer has
129 increased by ca. 1.3 °C and rainfall amounts have decreased in the months October to May
130 by average total of 67 mm (period 2017-2022 vs. 1994-2022, own climatic data recorded on-
131 site). The fallow period in the year before and the full winter wheat growing season 2021-2022
132 in the vegetative period (October to sample taking at flowering stage in May) did not exhibit a
133 large total precipitation deficit (+99 mm and -13 mm, for the periods respectively) as compared
134 to the averages of the previous years (2017-2022). However, some months in the period
135 January to May of 2022 were much drier as compared against the average 2017-2022 (total
136 14 mm vs. 39 mm). Indeed, the soil moisture in this experiment was extremely low at ca. 2.7%,
137 where another study by Santin-Montanya et al., (2020) reported around 10% at wheat flowering
138 stage for the years 2014 to 2016.

139 2.2 Experimental design

140 The three field tillage treatments consist of standard/conventional tillage (ST),
141 minimum/reduced tillage (MT), and no tillage (NT) and are arranged in a randomized complete
142 block design (4 blocks = treatment replicates) with 5 split-plots within each block of which four
143 contain crop rotations (Fallow, winter wheat, vetch, barley) and the fifth containing a wheat
144 monoculture. In the crop rotation before winter wheat, one year of bare fallow is employed to

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145 accumulate rainwater for subsequent wheat cropping. The tillage practices for ST consist of
146 mouldboard ploughing (30 cm), chisel (non-inversion) ploughing (20/15 cm) for MT, and no
147 tillage for NT with herbicide treatments when needed for weed control middle of May and
148 always at the beginning of October before sowing. Further details on seeding, nutrient, pest,
149 weed, and residue management, as well as crop performance, soil physico-chemical, and
150 climate aspects on this long-term trial were reported in preceding studies (Gandía et al., 2021,
151 Santin-Montanya et al., 2020, 2017, 2016, 2013, Tellez-Rio et al., 2017, Guardia et al., 2016,
152 Martin-Lammerding et al., 2015, 2013, 2011, Martin-Rueda, et al., 2007).

153 ### 2.3 Field sampling and sample processing

154 Shoots, roots and soil from the split-plots of winter wheat (*Triticum aestivum* var. Marius) in
155 crop rotation were sampled at flowering at the end of May 2022. Rhizosphere soil for the
156 analysis of soil microbial communities (bacteria, fungi), microfauna (nematodes), and
157 mesofauna (acari) was sampled as composite sample of five samples per plot. First, plants
158 were cut at the crown and then a shovel was used to extract the root system and adjacent soil
159 from an area diameter of 15 cm at two depths 0-10 cm and 10-20 cm. Nematodes were
160 sampled from a combined depth of 0-20 cm only. The soil at the center of the extracted root
161 system was shaken off and transferred to plastic bags for subsequent DNA and soil functional
162 analyses. For mesofauna, samples were carefully taken at the edges of the sampling holes as
163 intact pieces of soil blocks with a total volume of 250 ml per plot and kept in sturdy plastic
164 containers to avoid structural disturbance. For nematodes, a combined sample of 200 g soil
165 per plot was taken and kept in sealed plastic bags. In between wheat rows, 10-20 additional
166 soil samples were taken from 0-20 cm depth and combined to obtain 2 kg per plot for soil
167 chemical analyses. For analysis of root system architecture, two additional samples were taken
168 per plot: One root sample was taken with a precise volume (h=20 cm, d=9 cm) using a
169 pneumatic soil corer (Royal Eijkelkamp B.V, The Netherlands) to determine root length and
170 other quantitative parameters of root system architecture within that soil cylinder and an
171 additional sample was taken from the top 10 cm extracting the root crown to count first to third
172 order lateral roots and measure the respective root angles. For each root sample,

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173 corresponding aboveground plant biomass was sampled and kept in paper bags for drying at
174 room temperature. All soil and root samples were immediately cooled to 4°C and transported
175 to the lab. For further analysis, soil samples were sieved at 2 mm and stored at 4°C for
176 MicroResp® analyses or frozen at -20°C for DNA and enzymatic anaylses.

177 Root samples were carefully washed with water over 1 mm sieves to separate roots from soil.
178 Clean roots were subsequently stored in 70% ethanol. For quantitative root traits such as total
179 root length and network area, the washed root samples were transferred to petri dishes and
180 arranged without overlap of roots. Samples were scanned (Epson flatbed, 600 DPI, Seiko
181 Epson Corporation, Japan) and images were subsequently processed in RhizoVision Explorer
182 (Seethepalli et al., 2021). The root crown samples were digitized using Nikon D3400 (Nikon,
183 Tokyo, Japan) and images were processed in RhizoVision Explorer (Seethepalli et al., 2021)
184 and phenotyped for root angles and lateral root growth (Trachsel et al. 2011). Afterwards, root
185 samples were dried at 40°C in paper envelopes and root dry mass was determined.
186 Corresponding aboveground biomass was separated into leaves, shoots and heads and dry
187 weight determined.

188 ## 2.4 Soil chemical analysis

189 Soil gravimetric moisture content was determined by drying over night at 105 °C. Soil C and N
190 (%) were determined on a Vario Max Cube (Elementar Analysensysteme GmbH,
191 Langenselbold, Germany). Further soil elements were determined on inductively coupled
192 plasma optical emission spectroscopy (ICP-OES, Agilent 5110, Agilent Technologies Inc,
193 USA) with extractants as detailed in the following. $CaCl_2$ extraction matrix was used for pH
194 determination and estimation of plant available Mg. Calcium Acetate Lactate (CAL) extraction
195 matrix was used for reflecting plant available K and P (VDLUFA 2016, Scherer and Weichmann
196 (1991). Calcium chloride/DTPA (CAT) extraction matrix was used for estimating plant available
197 Cu, Fe, Mn, P, and Zn (VDLUFA 2008). Nitric acid and hydrochloric acid (Aqua regia (AR)/
198 King`s water) digestion was applied to determine total B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn
199 concentrations in soil (VDLUFA 1991). B concentrations were below the detection limit of 10
200 mg kg^{-1}.

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201 ## 2.5 Soil micro- and mesofauna analyses

202 Acari and Collembola were extracted from 500 ml of soil using Berlese-Tullgren funnels with
203 LED lamps (non incandescent 7 W bulbs of 600 lumens) for 15 days at constant temperature
204 (23 °C) and stored in 70% ethanol. To identify the collected specimens to family level,
205 dichotomous keys were used, e.g. Krantz & Walter (2009) for Acari and Jordana & Arbea
206 (1989) for Collembola. The specimens were preserved in 70% ethanol or mounted in semi-
207 permanent slides with Hoyer's medium, and have been deposited in the "Mesofauna collection"
208 at the Arid Zones Experimental Station (EEZA-CSIC). Only 4 specimens of Collembola were
209 collected in total and we will therefore omit them from further discussion. To assign mite
210 families to trophic guild (predator, fungivore, herbivore) the information in Krantz & Walter
211 (2009), in addition to expert knowledge (e.g. shape of the mouth parts), were considered.

212 Nematodes were extracted by a modification of wet sieving and the Baermann funnel method
213 (Barker, 1984). All nematodes were counted under a dissecting microscope and at least 100
214 individuals were identified to genus level under a light microscope after Bongers (1990).
215 Nematode abundances were expressed as number of individuals 100 g fresh soil^{-1}. Nematode
216 genera were classified into five trophic groups (bacterivores, fungivores, herbivores,
217 omnivores, and predators) after Yeates (1994), and into the colonizer-persister (cp) scale, that
218 classifies nematodes into five cp groups (cp1-5). Nematodes in low cp groups (cp 1-2) are
219 commonly r-strategist bacterivores and fungivores with high reproduction rates, short life
220 cycles, and resistance to environmental perturbation, while high cp groups (cp 3-5) are K-
221 strategists with progressively longer life cycles, lower reproduction rates and higher sensitivity
222 to perturbation and environmental stress (Bongers, 1990). Based on such functional
223 classifications, nematode participation on nutrient cycling was assessed through the use of
224 three nematode-based indices (Ferris, et al., 2001; Ferris, 2010). The Enrichment Index (EI) is
225 based on the relative abundances of bacterivores with low cp value which are enrichment-
226 opportunists, and is used as an indicator of fast organic matter decomposition mediated by
227 bacteria and active nutrient cycling. The Channel Index (CI) is based on the ratio fungivores to
228 opportunistic bacterivores and is used as an indicator of slow organic matter decomposition

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229 mediated by fungi. Both the EI and the CI range from 0 (no enrichment opportunists (EI) or
230 fungal feeders (CI) present in the community) to 100 (absolute dominance of such functional
231 groups). Finally, the Enrichment Footprint (EF) is a calculation of the potential amount of C
232 mineralized (µg C kg^{-1} soil) by enrichment opportunistic nematodes and thus an indicator of
233 the magnitude of the participation of such nematodes in the nutrient mineralization soil service.

234 2.6 Molecular microbial analysis

235 2.6.1 DNA extraction

236 DNA was extracted from 350 mg fresh weight soil using the Macherey Nagel NucleoSpin 96
237 Genomic DNA soil kit with the enhancer solution following the manufacturers
238 recommendations. Prior to extraction, samples were homogenized twice for 5 min in a
239 TissueLyser (Qiagen, Hilden, Germany) at 30 Hz in two different orientations and stored at -
240 20 °C. Extract quality was checked with Nanodrop 260/230 and 260/280 nm absorption and
241 on 1.5% agarose gel electrophoresis. DNA concentration was quantified with Qubit dsDNA HS
242 Assay Kit on 384 well plate reader (Tecan 200 Pro M Nano+).

243 2.6.2 Quantitative PCR

244 DNA extracts were diluted 10-times for quantitative PCR in a CFX384 Real-Time System (Bio-
245 Rad Laboratories, CA, USA). Prokaryotic 16S marker gene quantification was performed in
246 triplicate PCR reactions of 15 µL consisted of 7.5 µL 1x KAPA SYBR Fast qPCR Kit Master
247 Mix 2× Universal (Axonlab, Baden, Switzerland), 1.8 µL of each 10 µM forward and reverse
248 primer (BactQuant, Liu et al. 2012) and 2 µL of DNA template. Reaction conditions consisted
249 of an initial denaturation at 95 °C for 3 min, and 39 cycles of denaturation at 95 °C for 15 s,
250 annealing at 62 °C at 15 s, with final extension at 72 °C for 30 s. Melt curve analysis was
251 performed from 65-98 °C. The amplification efficiency was between 68.9%-70.6% and R^2 99.7-
252 99.8%. Prokaryotic copies were quantified against a ten-fold dilution series in triplicate of a 465
253 bp long target region on pJET 2974 plasmid (CloneJET PCR Cloning Kit, Thermo Fisher
254 Scientific, Switzerland). Eukaryotic/fungal 18S marker gene quantification was performed in
255 triplicate PCR reactions of 15 µL consisted of 7.5 µL 1x KAPA SYBR Fast qPCR Kit Master
256 Mix 2× Universal (Axonlab, Baden, Switzerland), 0.75 µL of each 10 µM forward and reverse

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257 primer (FR1 and FF390, VAINIO and HANTULA, 2000) and 2 µL of DNA template. Reaction
258 conditions differed to the bacterial qPCR in that 35 cycles were carried out with annealing at
259 50°C. The amplification efficiency was between 92.8%-95.9% and R^2 99.6-99.9%. Eukaryotic
260 copies were quantified against a ten-fold dilution series in triplicate of a 390 bp long target
261 region on pJET 2974 plasmid (CloneJET PCR Cloning Kit, Thermo Fisher Scientific,
262 Switzerland).

263 ### 2.6.3 Metabarcode analysis of bacterial and fungal community

264 Bacterial and fungal amplicon libraries were performed in a limited cycle PCR amplification
265 approach followed by an Illumina barcode indexing PCR for sample pooling (Illumina 16S
266 Metagenomic Sequencing Library Preparation, Genetic Diversity Center ETH Zürich). For
267 amplification of the 16S V3-V4 target region, primers 341F (5´- CCTAYGGGDBGCWSCAG-
268 3') and 806R (5'-GGACTACNVGGGTHTCTAAT-3') (Frey et al., 2016) were used with added
269 nextera and sequencing adaptors. The fungal ITS2 target region enclosed by the 5.8S and
270 28S gene cassette was amplified with ITS3ngs (5'-CANCGATGAAGAACGYRG-3') and
271 ITS4ngs (5'- CCTSCSCTTANTDATATGC-3') (Tedersoo and Lindahl, 2016) and the same
272 adaptors as used for bacteria. The first PCR was performed in triplicate PCR reactions of 15
273 µL consistent of 7.5 µL 1x KAPA SYBR Fast qPCR Kit Master Mix 2× Universal (Axonlab,
274 Baden, Switzerland), 0.4 µL each of 10 µM forward and reverse primers, and 2 uL of DNA
275 template of different DNA template dilutions (1:5, 1:10, 1:25 for bacteria and 2 x original
276 concentration and 1:5). The reaction conditions for bacterial and fungal sets consisted of initial
277 denaturation at 95 °C for 3 minutes, followed by 35 cycles (bacteria) or 40 cycles (fungi) of
278 denaturing 95 °C for 20 s, annealing 58 °C (bacteria) and 60 °C (fungi) for 15 s and extension
279 72 °C for 40 s and a final extension at 72 °C for 10 min. Triplicate reactions were pooled and
280 purified using a magnetic bead solution (https://openwetware.org/wiki/SPRI_bead_mix) and
281 visualized on agarose gel (1.5%). The indexing PCR step was carried out as per default
282 instructions of the Ilumina indexing kit where the template concentration was prediluted to 0.1-
283 0.5 ng DNA µL-1. Index PCR reactions were purified using a magnetic bead solution
284 (https://openwetware.org/wiki/SPRI_bead_mix), visualized on agarose gel (1.5%) and

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285 quantified with Qubit dsDNA HS Assay Kit on a 384 well plate reader (Tecan 200 Pro M Nano+)
286 before library pooling. Final libraries were quantified and size checked with TapeStation (4150
287 TapeStation System, Agilent Technologies, Switzerland). Both libraries including non-template
288 PCR/library control (n=1) and processing blanks (n=2) were sequenced separately on Illumina
289 MiSeq v3 (Illumina Inc., San Diego, CA, USA) paired end 2x300 bp sequencing at Genome
290 Quebec CES (Montreal, Canada) with ~7% PhiX per sequencing run.

291 Sequencing reads were prepared at Genetic Diversity Center (GDC), ETH Zürich, in the
292 USEARCH (Edgar, 2010) pipeline. Reads were demultiplexed, PhiX and low complexity
293 samples were removed, and reads trimmed at each end with 30 or 55 bp length. Read pairs
294 were synchronized, merged with minimum 30 bp overlap, 100 bp length, and 60% identity, and
295 primer sites removed in-silicio. Size and quality of amplicons was selected at 300-500 bp length
296 for 16S and between 200-500 bp for ITS2 with minimum mean Q score of 20 leaving 6.4 mil.
297 (5.5 mil. unique) and 11.2 mil. (1.7 mil. unique) amplicons for 16S and ITS2 sets, respectively.
298 UNOISE3 was used to deduplicate amplicons and denoise sequences with zero radius
299 operational taxonomic units clustered de-novo at 97% similarity (97% zOTUs). Amplicons were
300 backmapped to OTUs (counting). Taxonomy assignment was made with SINTAX for 16S
301 against SILVA SSU v138 (RESCRIPt) (Quast et al., 2012) with tax filter at 0.75. For ITS2,
302 taxonomy assignment was made against UNITE Eukaryotes USEARCH/UTAX release v83
303 (Nilsson et al., 2019) with tax filter at 0.75. Additionally, ITSx (Johan Bengtsson-Palme et al.,
304 University of Gothenburg Version: 1.1.3) was used to extract and identify the ITS region. Reads
305 that were not classified as fungi based on ITSx were removed prior to further cleaning detailed
306 in the next section. Cleaning of non-target taxa and contaminants was done on the whole set
307 before splitting into the field experiments. For the 16S data, archaea, mitochondria and
308 chloroplasts were removed. All taxa (n=13) found in NTC and blanks were removed from
309 analysis. The average bacterial read count was 18,355 reads, minimum reads at 7,793,
310 consisting of 1,666 taxa, and with a data sparsity of 35%. For the ITS2 dataset, all phyla other
311 than fungi and taxa found in NTC and blanks (n=214) were removed. On average 14,012 reads
312 remained with a minimum at 7,042 reads of 1,277 taxa with a data sparsity of 75%.

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313 ## 2.7 Soil functionality

314 Activities of seven hydrolytic extracellular enzymes were measured, including four enzymes
315 involved in C-acquisition: α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylanase; two
316 enzymes involved in N-acquisition: leucine aminopeptidase, β-N-acetylglucosaminidase, and
317 one enzyme involved in P-acquisition: phosphatase. Extracellular enzyme activity (EEA) was
318 assessed using standard fluorometric techniques and the activities, represented by nmol g^{-1}
319 dry soil h^{-1}, of the C, N, and P acquiring enzymes were grouped to represent the general
320 potential C, N, and P acquisition activity in soil samples (Bell et al., 2013).

321 Chemotrophic, respiratory soil CO_2 evolution upon addition of different substrates was
322 determined with Microresp^{TM} system (Campbell et al., 2003). Absolute respiration rates (μg
323 CO_2–C g^{-1} dry soil eq. h^{-1}) were calculated as shown in the MicroResp™ technical manual
324 (Cameron, 2007). For each sample, eight technical replicates were measured and obvious
325 outliers removed (coefficient of variance > 20). In addition, reference soils were included as
326 standards in each run (Creamer et al., 2016). Soils were pre-incubated for one week at the
327 later assay temperature and soil basal respiration was measured as soil samples
328 supplemented with deionized water, and multiple substrate-induced respiration (MSIR) as the
329 total CO_2 flux from all substrates (keto-glutaric acid, oxalic acid, xylan, glucose, N-acetyl-
330 glucoseamine, alanine, amino-butyric acid). Microbial biomass (SIR-MBC) was estimated from
331 the glucose (ca. 59 mg g^{-1} soil dry weight eq.) induced average respiration rate volume after 5
332 h of incubation and using the formula from Anderson and Domsch (1978). The metabolic
333 quotient (qCO2) was calculated as basal respiration over SIR-MBC (Wardle and Ghani, 1995,
334 Anderson and Domsch, 2010).

335 ## 2.8 Wheat harvest analysis

336 At maturity, grains were harvested using a plot harvester. Grain yield was assessed after
337 cleaning and air drying the grains. Thousand-kernel weight was measured with an Opto-Agri
338 (Optomachine, Riom, France). For mineral nutrient analyses in wheat grains, dried and milled
339 samples were mineralized by microwave (UltraClave V Fa. MLS Leutkirch, Germany) digestion
340 with HNO_3. (VDLUFA, 2021). In samples where silicon was to be analyzed, a second

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341 microwave digestion was performed and subsequently 0.5 ml HF were added to inhibit
342 polymerization and precipitation of the dissolved silica. Grain nutrient analysis of C, N, S was
343 determined via Elemental-Analyzer (Elementar Vario EL, Elementar Analysensysteme GmbH,
344 Langenselbold, Germany; VDLUFA, 2012), Cl on Ion Chromatography (Integrion, Thermo
345 Fisher Scientific, Waltham, MA, USA; VDLUFA, 2019), Ca, Cu, Fe, K, Mg, Mn, Na, P and Si
346 on inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 5110, Agilent
347 Technologies Inc, USA), Co, Mo, Ni, and Se on ICP-MS (Perkin Elmer NexION 300 X,
348 PerkinElmer, Waltham, MA, USA), and I with ammoniacal extraction on ICP-MS (Perkin Elmer
349 NexION 300 X, PerkinElmer, Waltham, MA, USA). All analyses were conducted at Core Facility
350 University of Hohenheim, Analytical Chemistry Unit. All Na and I was below the detection limit
351 at 20 mg kg-^{1} or 0.5 mg kg^{-1}, respectively. Most values of Se were below detection limit at
352 0.025 mg kg^{-1}. For Si and Co some values were below detection limit and were replaced with
353 half detection limit value at 150 mg kg^{-1}and 0.025 mg kg^{-1}, respectively.

354 Analysis of seven grain B vitamins was performed as reported previously (Cao et al., 2024).
355 Briefly, around 50 mg of homogenized wheat seeds were extracted with 1 mL of 50 mM
356 phosphate buffer containing internal standards for each type of B vitamin. The extract was
357 treated with multi-enzyme incubation to release protein-bound forms and associated bioactive
358 derivatives and analyzed with liquid chromatography coupled with tandem mass spectrometry
359 (UHPLC-MS/MS).

360 Technological properties of wheat grains included the distribution of starch size classes
361 distinguishing between A-type (>10 µm), B-type (2-10 µm) and C-type (<2 µm) starch and grain
362 glutenins and gliadins contents. The size distribution of the purified starch granules was
363 estimated with a Mastersizer 2000E laser particle size analyzer and the Hydro 2000SM small
364 volume wet sample dispersion unit (Malvern Panalytical Ltd, Palaiseau, France) following the
365 protocol reported by Edwards et al (2008). Protein composition was determined after
366 sequential extraction. Briefly, 66.6 mg fresh weight of complete flour were mixed with 1 mL of
367 50 mM phosphate buffer (pH 7.8) containing 100 mM NaCl (30 min, 800 rpm, 4 °C). After
368 centrifugation (10 min, 18,000 g, 4 °C), the pellet was mixed with 1mL of the same 50 mM

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369 phosphate buffer (10 min, 800 rpm, 4 °C) and centrifuged in the same condition. This operation
370 was repeated once. To extract gliadins the pellet was mixed with 1 mL 70% ethanol (30 min,
371 1100 rpm, 4 °C). After centrifugation (10 min, 18,000 g, 4 °C), extraction was done with the
372 same 70% ethanol buffer (10 min, 1100 rpm, 4 °C) and centrifuged. This operation was
373 repeated twice. After each centrifugation the supernatant containing gliadins was collected and
374 pooled. Finally, to extract glutenins the resulting pellet was mixed with 0.5 mL of 25 mM borate
375 buffer (pH 9.8) containing 50 % propanol-2 and 1% DTT (30 min, 1200 rpm, 50 °C) and
376 centrifuged (10 min, 18,000 g, 18 °C). This operation was repeated once. After each
377 centrifugation the supernatant containing glutenins was collected and pooled. For stability,
378 glutenin fraction was alkylated with 4.6% of 4-Vinylpyrridine (15 min, 60 °C). The two glutenin
379 subunits (High-Molecular Weight Glutenin Subunit, HMW-GS; Low-Molecular Weight Glutenin
380 Subunit, LMW-GS) and four gliadin classes (α/β-, γ-, ω1,2-, ω5-gliadin) were quantified by
381 reverse phase high performance liquid chromatography (RP-HPLC) using an Agilent 1290
382 Infinity LC system (Agilent Technologies, California, USA) as described by Dai et al. (2015).
383 Briefly, gliadin and glutenin extracts were filtered through regenerated cellulose syringe filters
384 (0.45-µm pore diameter, UptiDisc, Interchim, Montluçon, France), then 4 µl of each protein
385 extract were injected into a C8 reversed-phase ZORBAX 300 Stable Bound column (2.1 × 100
386 mm, 3.5 µm, 300 Å; Agilent Technologies) maintained at 50°C. Proteins were separated at a
387 flow rate of 1 ml/min using linear solvent gradients from 24 to 50% acetonitrile containing 0.1%
388 (v/v) trifluoroacetic acid over 13 min for gliadins, and from 23 to 42% over 25 min for glutenins.
389 Proteins were detected by UV absorbance at 214 nm. Chromatograms were processed with
390 ChemStation 10.1 software (Agilent Technologies) and the HPLC peaks corresponding to each
391 of the four gliadin classes and the two glutenin subunits were identified following the
392 observations of Wieser et al. (1998; Figure S4). All quantities were corrected to take into
393 account the extraction yield which was estimated to be 93% for all fractions. In addition, the
394 glutenin quantities were then corrected to take into account the extraction yield without SDS
395 which was estimated to be 70% (Nicolas et al 1998). Protein quantities were determined with

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396 a calibration curve based on the Dumas combustion method using a FlashSmart N Analyzer
397 (ThermoScientific, Villebon-sur-Yvette, France).

398 ## 2.9 Statistical analyses

399 For data handling, descriptive analysis, and plotting *R* version 4.3 (R Core Team, 2023) was
400 used with tidyverse, grid and gridExtra packages (Wickham et al. 2019, RCore team 2023,
401 Auguie, 2017). Hulls were drawn with ggConvexhull (Martin, 2017) and labels with ggRepel
402 (Slowikowski, 2023).

403 Treatment effects on univariate data (soil chemical, wheat traits, organism abundance,
404 Shannon diversity, and functional activity) were tested by ANOVA with linear model of block
405 and management as fixed factors when from one/composite depth. The models for ratio of
406 eukaryotic to prokaryotic qPCR values and root to shoot ratio were tested as ANCOVA with
407 nominator as response and denominator as covariate. Values were Box-Cox transformed
408 when multiple DHARMa diagnostics (Hartig, 2022) indicated violations. Models that still failed
409 in DHARMa diagnostics are reported in brackets. Fixed factors were tested with type III
410 ANOVA. With data from two depths, (Generalised) Linear Mixed effect Models (GLMM, GLM,
411 or LMM) were used as appropriate for type of response variable with block, management,
412 depth, and management:depth interaction as fixed factors and plot as random factor. The
413 metabolic quotient as the ratio of basal respiration over biomass was modelled as LMM in glm
414 function with denominator as B-spline in splines package (R core team, 2023). For acari count
415 data, GLMM with negative binomial family was used after comparing Akaike`s information
416 criterion with Poisson family model. For LMM models, fixed factors were tested with type III
417 ANOVA, Kenward-Roger F-test and Satterthwaite's degrees of freedom. For GLM and GLMM
418 models, fixed factors were tested with type II ANOVA and Likelihood Ratio test or Wald Chi
419 square test, respectively. For all models, values reported are estimated marginal means from
420 linear combination of model coefficients and were back-transformed for reporting. Brackets
421 report the 95% confidence interval (CI). R packages used were base stats, spline, lme4,
422 glmmTMB, lmtest, DHARMa, emmeans, car & effects, and multcomp (RCore team 2023, Bates

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423 et al., 2009, Brooks et al., 2017, Zeileis and Hothorn, 2002, Hartig, 2022, Lenth, 2023, Fox and
424 Weisberg, 2019, Hothorn et al., 2008).

425 For amplicon sequencing data, rarefaction was made to the minimum sequencing depth
426 sample (Schloss, 2024) with vegan`s rrarefy (Oksanen et al., 2022). Rarefaction with 999
427 repeats was used for repeatedly calculating alpha and beta diversity indices for averaging over
428 sub-sampling results (Cameron et al., 2021). Beta diversity (site dissimilarity) for bacteria,
429 fungi, acari and nematode data was calculated with Bray-Curtis index (Bray and Curtis, 1957).
430 Alternatives such as e.g. centered-log ratio transforms and Aitchison`s distance to address
431 compositionality (Gloor et al., 2017, Zhou et al., 2022), were also checked (not shown), but
432 were argued to be unnecessary to control type I error of community-wide tests (Zhou et al.,
433 2022). Homogeneity of multivariate group dispersions were tested with betadisper test
434 (Anderson, 2006) in vegan at 5% significance level. Clustering with unweighted pair-group
435 method arithmetic average (UPGMA) and ordination of sample to sample dissimilarities were
436 done with Principal Coordinate Analysis (PCoA) in base R cmdscale function, respectively.
437 Significance of tillage and depth factors (incl. interactions) on community dissimilarity were
438 determined with the permutational multivariate ANOVA (perMANOVA) (Anderson, 2001) in
439 vegan with 9999 permutations and restricting permutations to block when block was found to
440 be significant at $p < 0.05$.

441 Differential abundance of bacterial and fungal taxa was determined with LinDA (Zhou et al.,
442 2022) at 5% FDR on the rarefied dataset and using centered log-ratio transform (Yang and
443 Chen, 2023, 2022). Combinations of depth and tillage, and block were considered as fixed
444 factors and plot as random factor. Contrasts with significantly differential abundant taxa were
445 retrieved from model coefficients in LinDA Wald test with multiple test correction by the
446 Benjamini-Hochberg method. In this dataset, especially for the sparse fungal set, the LinDA
447 method reported conservative positive detections among other tools employed, such as for
448 example DESeq2 (Love et al., 2014) or per taxon application of glmmTMB (Brooks et al., 2017)
449 (not shown). For cluster analysis, significant taxa were gathered and their log2 transformed

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450 counts (+1 pseudo-count) subtracted from their global mean, Euclidean distances calculated,
451 and hierarchically clustered with Ward's rule (Love et al., 2014). Heatmaps were drawn in
452 pheatmap package (Kolde, 2019) and the number of informative "change" clusters were
453 identified with Gap statistic (b=999 folds) and the Tibshirani et al. (2001) criterium in cluster
454 package (Maechler et al., 2022, Akalin, 2020). The silhouette (similarity) value (Rousseeuw,
455 1987) of clusters and taxa to their peers was calculated (Akalin, 2020).

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456 # 3. Results

457 ## 3.1 Effects of tillage intensity on soil properties

458 Soil chemical and physical properties were analyzed to assess the effect of tillage intensity on
459 soil properties after running for almost 30 years. Results showed that total C and N in both
460 depths sampled (0-10 and 10-20 cm) were twice as large in NT as compared to ST and MT
461 ($p<0.05$, Table 1). Similarly, significantly highest concentrations of total soil P (P_{AR}) and plant
462 available K (K_{CAL}) were found in NT ($p<0.05$, Table 1). Concentrations of other macro- and
463 micronutrients were similar across tillage treatments (Table 1), thus data for plant available
464 Cu, Fe, Mn, P, and Zn measured using Calcium chloride/DTPA (CAT) extraction are not
465 shown, but are available on Zenodo (link will be added during revision process). Soil moisture
466 contents were low in all treatments ranging between 2.6 to 3.2% with significantly lowest values
467 in NT compared to MT and ST ($p<0.05$).

468 ## 3.2 Impact of tillage intensity on soil biodiversity

469 To assess the impact of tillage intensity on soil biodiversity, a range of biological indicators
470 based on molecular and classical morphological assessments were analyzed. Reducing tillage
471 intensity (MT and NT) significantly increased the abundances of gene copy numbers of
472 prokaryotes and eukaryotes as compared to ST ($p<0.05$, Table 2). The ratio of eukaryotic to
473 prokaryotic gene copies in topsoil increased on average with reduction of tillage intensity from
474 0.12 in ST, over 0.13 in MT, to 0.15 in NT. Acari were found to be on average most abundant
475 in NT. In particular, predatory acari were significantly most abundant under NT (Table 2). Of
476 note was that total and fungivore acari were significantly more abundant in ST than in MT.
477 Nematode abundance did not differ significantly across tillage treatments. On average, total
478 abundance of nematodes, including their trophic groups such as bacterivores, fungivores, and
479 herbivores increased consistently from ST over MT to NT (Table 2). This trend parallels the
480 increase in prokaryotic and eukaryotic gene copies (Table 2). Regarding community richness
481 and evenness of the respective communities, as assessed by the Shannon index, the bacterial
482 diversity was found to be significantly reduced in NT ($p<0.05$), while the diversity of other
483 communities did not differ significantly with tillage intensities (Table 2).

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484 Multi-taxa community composition dissimilarities as calculated using the Bray-Curtis index
485 were ordinated by Principal Coordinate Analysis (PCoA) for bacteria, fungi, acari and
486 nematodes. This showed in 2D space a gradient along with tillage intensity (Figure 1 A-D).
487 Permutational ANOVA supported this finding by indicating significant effects of tillage intensity
488 ($p<0.05$) on bacterial, fungal and mite communities, but not on nematodes (Table A1). Soil
489 depth was not found to be a significant factor in community composition.

490 Taking a closer look at the structure of bacterial communities across all treatments,
491 Planctomycetota constituted the relatively most abundant phylum at 39.0% followed by
492 Acidobacteria (15.8%), Actinobacteriota (14.4%), Proteobacteria (8.6%), Chloroflexi (8.5%),
493 and Verrucomicrobiota (7.9%) (Figure A1). Besides Cyanobacteria, which showed the most
494 pronounced tillage-induced shifts in relative abundance increasing from 0.5% under ST and
495 MT to 3.8% under NT, several other taxa also changed (E-supplementary B1-B9).

496 Differential abundance analysis showed that 136 bacterial taxa were significantly associated
497 with tillage and only one with depth (LinDA, FDR<5%). These candidates represented the
498 maximum of 23% (NT, 0-10 cm) and minimum of 17% (MT, 10-20 cm) of 16S rRNA gene
499 counts. The change profiles of these bacteria clustered according to Gap statistic and
500 Tibshirani criterion into seven informative groups (Figure 2A). Clusters 7, 6 and 3, in order of
501 decreasing mean log-fold change, were characterized by relative increases of bacteria under
502 NT. A taxon from the genus *Tychonema* (order Cyanobacteriales) in cluster 7 was particularly
503 prominent in terms of high relative abundance at 3.5% at 0-10 cm depth and 3.3% at 10-20
504 cm depth under NT and its high relative abundance when compared to ST and MT (Figure 2A
505 cluster 7, Figure A37). The cluster 3 contained particularly relatively abundant taxa. These
506 were taxa such as a Rhizobium from the genus *Microvirga* (family *Beijerinckiaceae*),
507 Actinobacteria from the family *Micromonosporaceae* and *Blastocatellaceae*, and
508 Planctomycetes from the Tepidisphaerales, and Isosphaerales.

509 Bacteria with the highest relative abundance under ST, and/or MT were placed in clusters 1,
510 2, 4, and 5 (Figure 2A, Figure A37). Particularly interesting in the context of plant responses

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511 was cluster 4 with significant enrichment of taxa from *Bacillus*, *Cohnella*, and *Streptomyces*
512 under ST.

513 Taking a look at fungal community structure derived from PCR amplified ITS2 barcoding, the
514 most relatively abundant phyla in descending order were Ascomycota (44%), Basidiomycota
515 (25%), Chytridiomycota (5%), Mortierellomycota (4%), Glomeromycota (3%), Mucoromycota
516 (1%), and Monoblepharomycota (0.3%) (Figure A38). Further, more basal fungal clades were
517 also detected in lower relative abundance such as Aphelidiomycota, Basidiobolomycota,
518 Olpidiomycota, Rozellomycota, and Zoopagomycota (E-supplementary F1-F9).

519 Differential abundance analysis indicated that 51 fungal taxa were significantly associated with
520 tillage and only one with depth (FDR<5%). They represented a maximum of 32% (NT, 0-10
521 cm) and a minimum of 8% (MT, 0-10 cm) of the ITS2 sequences. The change profiles of fungi
522 clustered into 6 informative groups (Figure 2B, Figure A63). Cluster 5, 1, and 2, in order of
523 decreasing mean log-fold change, were associated with relative increases of taxa under NT
524 (Figure 2B, Figure A63). In particular, *Coprinellus curtus* (order Agaricales, phyl.
525 Basidiomycota) in cluster 5 exhibited a strong enrichment in topsoil, increasing from 0.004%
526 under ST to 20.6% under NT conditions. Similarly, other Agaricales in cluster 1 and 2 including
527 *Psathyrella* (fam. *Psathyrellaceae*), *Crepidotus* (fam. *Crepidotaceae*), and *Conocybe* (fam.
528 *Bolbitiaceae*) increased under NT. Other taxa in cluster 5 were from the phylum Ascomycota
529 and the classes Dothideomycetes (fam. *Didymosphaeriaceae* and *Phaeosphaeriacea*) and
530 Leotiomycetes (fam. *Sclerotiniaceae*), amongst others (Figure A63). Clusters 3, 4, and 6 were
531 characterized by strong relative decreases of fungi under NT (Figure 2B). Particularly cluster
532 3 contained relatively abundant fungal taxa, such as *Zymoseptoria triticii* (Ascomycota),
533 *Aspergillus thesauricus* (Ascomycota), *Ascobolus* (Ascomycota), *Udeniomyces*
534 (Basidiomycota) and several unassigned Ascomycotan families. Cluster 4 was represented by
535 taxa with strongest relative increases under MT, containing a taxon from the genus *Fusarium*
536 (Ascomycota), and further assigned taxa from the same class such as *Achaetomium*, and
537 *Metacordyceps*.

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538

539 ### 3.3 Impact of tillage on soil functionality

540 Soil functionality was assessed by community level substrate utilization profiling (CLPP),
541 potential enzymatic activity (EEA) of C, N, and P compounds, and calculations of C flow
542 through the nematode community (Table 3). Tillage treatment significantly affected CLPP and
543 EEA levels ($p<0.01$). Values for CLPP were significantly higher in NT and MT vs. ST ($p<0.05$),
544 and for the former treatments it was also found that in top soil (0-10 cm) significantly higher
545 values were measured vs. lower depth (10-20 cm). EEA of C, N, and P cycles was significantly
546 higher in NT vs. MT and ST ($p<0.05$). On average, NT exhibited in all soil functionality
547 measures the highest values, except for the fungal mediated OM decomposition derived from
548 nematode composition.

549 ### 3.4 Impact of tillage on wheat performance

550 At flowering stage, total aboveground biomass of individually weighed wheat plants was
551 significantly affected by tillage ($p<0.05$), decreasing from ST, over MT to NT by more than 2-
552 fold (Table 4). This trend was seen alike when resolving for the compartments head, leaves
553 and stem. The root biomass was not significantly different between tillage treatments at this
554 growth stage ($p>0.05$), however, on average, wheat under ST exhibited higher root biomass,
555 followed by NT and lowest under MT (Table 4). The root:shoot ratio was significantly higher
556 under NT compared to MT and ST ($p<0.05$). The wheat root-to-soil interface was resolved
557 further by characterizing root traits including number of root tips, branch points, total
558 length/area/volume, diameter of roots and root orientation (Table 4). Only root median
559 diameter was significantly higher under NT compared to ST and MT ($p<0.05$). Not significant,
560 but on average higher under NT were also root average diameter, root maximum diameter and
561 large (>0.2 mm) root diameter length. For wheat roots under ST, the number of root tips, root
562 surface area and total root volume were on average highest. And for wheat roots under MT,
563 number of root branch points, branching frequency, total root length, the proportion of root
564 length in fine (<0.1 mm) and medium (0.1-0.2 mm) range diameter classes were on average
565 highest, while root angle orientation was lowest (Table 4).

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566 At harvest, wheat yields and thousand kernel weight were similar across treatments (Figure
567 5). In contrast, grain N concentration (%) were significantly higher under ST compared to those
568 of NT ($F_{2,6}$=5.6, $p<0.05$, Table 5). In correspondence to the highest N content in grains grown
569 under ST, the grain B vitamin content for Thiamine (B1) and Riboflavin (B2) were found to be
570 significantly highest under ST compared to NT and MT. Interestingly, grain Ca and Si contents
571 were significantly highest in wheat grown under MT, while other macronutrients such as C, P,
572 K, S, Mg and micronutrients did not differ between tillage treatments. Regarding technological
573 grain properties, also the distribution of starch size classes significantly differed between
574 treatments with a higher abundance of A-type starches (>10 µm) in ST compared to NT ($p<$
575 0.01) and a higher abundance of B-type starches (2<x<10 µm) in NT compared to ST ($p< 0.01$)
576 (Table 5). In contrast, the abundance of gliadins and glutenins was similar across treatments
577 (Table 5).

578

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579 ## 4. Discussion
580

581 ### 4.1 Reducing tillage intensity enhanced soil chemical and biological properties
582 The increases in total soil C, N, P, and K under NT are consistent with previously reported
583 results of increased organic matter measures from the same field experiment (Martin-Rueda
584 et al., 2007, Martín-Lammerding et al., 2013, 2011), as well as the results of the meta-study by
585 Haddaway et al. (2017).

586 Unexpectedly, the soil moisture content in NT was the lowest (Table 1). This was not reported
587 in previous studies from the same site, where NT exhibited higher soil water content at
588 flowering stage. Indeed, it was argued that reduced tillage practices are also water saving
589 practices (Santin-Montanya et al., 2020). Further, a higher organic matter content would in
590 principle enable higher water holding capacity from aggregate formation and pore space. The
591 differing water status under NT in this study could derive from temporal water dynamics in
592 connection with sampling time. Co-occurring plants (e.g. weeds) were found to influence soil
593 water status by complex interactions with seasonal total rainfall, legacy effects over years, and
594 with short-term weather/sampling time (Gandia et al., 2021, Santin-Montanya et al., 2020).

595 Corresponding to the higher C content in NT, our results showed increased abundances of
596 prokaryotic and eukaryotic gene copies, and on average an increase in acari and nematodes
597 (Table 2) as well as higher SIR microbial biomass (Table 3). In addition, we observed higher
598 basal respiration, substrate utilization rates and enzymatic activities under NT (Table 3), which
599 are indicators for higher microbial biomass. Our results are consistent with the meta-analysis
600 of Chen et al. (2020) who found a positive effect of reduced tillage on the abundance of bacteria
601 and fungi as well as an increase in total C and N that might be linked with the increase of SOC
602 under NT (Lori et al. 2017). Similarly, a positive association of soil biological indicators with
603 decreasing tillage intensity was observed in the meta-analysis of Nunes et al. (2020). However,
604 despite that MT exhibited similar prokaryotic and eukaryotic gene copies to NT, the functional
605 activity levels were significantly lower (Table 3). This could result from significantly different
606 bacterial and fungal microbial communities (Figure 1, Figure 2), that would be expected to

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607 result in different functionalities and activity levels. Indeed, a previous study from the same
608 field experiment showed an increase in soil basal respiration, microbial biomass C and β-
609 glucosidase activity under NT (Martín-Lammerding et al., 2015). The observed increased in
610 eukaryotic:prokaryotic qPCR gene copy ratio (Table 2) from ST, over MT, to NT is consistent
611 with findings that reduced tillage practices are resulting in increased total microbial biomass
612 and fungal proportions (Six et al., 2006, Piazza et al. 2019). In contrast, van Groenigen et al.,
613 (2010) and Murugan et al., (2014) did not find a change in the proportion of bacteria and fungi
614 under reduced tillage, but an increase in saprotrophic fungi, which is in line with the increase
615 in relative abundance of taxa with saprotrophic/seed nutrition under NT observed in this study
616 (discussed in section 4.3). The studies of van Groenigen et al., (2010) and Murugan et al.,
617 (2014) highlighted the importance of differing organic matter degrading capabilities of fungal
618 groups and that this can have an effect on the organic matter dynamics. An increased
619 proportion of fungal decomposers could also be an alternative explanation, apart from stress
620 and disturbance (Wardle and Ghani, 1995, Anderson and Domsch, 2010), for the here
621 observed increase of the metabolic quotient from ST, over MT, to NT (Table 3).

622 4.2 Tillage intensity shapes soil biota community structure

623 As hypothesized, tillage intensity significantly shaped bacterial, fungal, and acarid
624 communities, while this could not be shown for nematodes (Figure 1, Table A1). Largely this
625 is consistent with previous studies documenting differences in the community structures of
626 microorganisms (Hartman et al., 2018; Kraut-Cohen et al., 2020; Orrù et al., 2021; Wang et
627 al., 2020), nematode (Puissant et al., 2021), and acari (Betancur-Corredor et al., 2022)
628 between ploughed, reduced tillage, and no-tillage systems. A key factor driving these
629 differences may be the increase in SOC with reduced tillage intensity and the associated
630 increase in microbial community size and activity (Lori et al. 2017, Ramírez et al., 2020).
631 Moreover, tillage intensity has been demonstrated to impact various soil properties, including
632 the stability and size of aggregates and bulk density (Li et al., 2019; Nunes et al., 2020; Mondal
633 and Chakraborty, 2022). These properties are also influenced by soil microbes, which in turn
634 might shape the other soil community by bottom-up forces (Hartmann and Six, 2022, Philippot

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635 et al., 2024). Since tillage intensity affects numerous relative abundance changes at the
636 microbial level, even contrasting within families and genera, an extensive ecological
637 description of bacterial and fungal clades with changes under tillage intensities can be found
638 in the supplementary (supplementary data 1.1.1 for bacteria and 1.4.1 for fungi). From a
639 biological standpoint, these findings align with ecological theory. Tillage-induced changes in
640 nutrient availability (quality and quantity of organic matter, root traits) and further soil properties
641 lead to shifts in multidimensional niche space. Indeed, the competitive exclusion principle
642 states that if two species use the exact same resources, the one using the resources more
643 efficiently will exclude the other (Gauss 1934). Therefore, coexistence of such a high number
644 of bacterial and fungal taxa (hundreds to thousands) found on a small volume that DNA was
645 extracted from, and with many taxa using similar resources is only possible, if substantial niche
646 differences evolve between closely related taxa or taxa using similar resources (the
647 multidimensional niche: Hutchinson, 1959). Of these niche dimensions a high degree of micro-
648 spatial separation and environmental variability could play a role (Horner-Devine et al., 2004).
649 Indeed, high resolution of soil bacterial translational dynamics have shown that competition
650 within nutritional guilds was strongest and affected by specialist-directed nutritional substances
651 and abundance of competitors (Moyne et al., 2023). For a more in-depth discussion, we will
652 focus on two key aspects: the formation of soil biocrust (explained later) under NT and the
653 dynamics of fungal plant pathogens and fungal antagonists.

654 Under NT, the most pronounced gene copy increase in bacteria was from a taxon in the
655 *Tychonema* genus (a Cyanobacterium) (Figure 2A, cluster 7). This taxon assignment is
656 monophyletic with *Microcoleus vaginatus* (Strunecky et al., 2023, Zhang et al., 2016), which
657 are well described for their importance to soil biocrust formation (Büdel, 2005, Powell et al.,
658 2015). Given the increase in total 16S gene copies under NT (Table 2), the increase in
659 cyanobacteria may hint at their role in soil C and N accumulation (Table 1). This could
660 contribute to soil biocrust formation, a less studied aspect in arable farming. Biological soil
661 crusts are linkages of mineral soil particles with a community of photo-autotrophic
662 cyanobacteria, algae, lichens, bryophytes, and associated heterotrophic fungi and prokaryotes

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663 that often develop in arid regions or on early habitats such as rocks. The organismal
664 composition is structured by local degree of abiotic stresses such as aridity and solar radiation
665 (Büdel, 2005, Grishkan et al., 2019, Hu et al., 2003). In arable farming, where the soil surface
666 is often disturbed and exposed when bare, particularly in semi-arid regions, the pioneering role
667 of biocrusts and microbial photoautotrophs is interesting in regard to their reported
668 contributions to SOM built-up, N_2-fixation, and climate resilience (Grishkan et al., 2019, Powell
669 et al., 2015, Maddigan, et al., 2013, Büdel, 2005, Hu et al., 2003, Mazor et al., 1996). Besides
670 *Tychonema*, we also detected other cyanobacteria some associated to lichens or algae (from
671 the order Chaetothyriales such as *Bradymyces*, *Exophiala*, Lücking et al., 2009), as well as
672 micro-fungal and bacterial heterotrophs that were reported to occur in soil biocrusts
673 (supplementary data 1.4). Thus, our results suggest that the development of biocrusts is
674 possible under NT, whereas ST and MT might exhibit a "critical" degree of disturbance (Figure
675 A65).

676 Focusing on fungal pathogen – antagonist interactions, we observed a relative decrease of
677 two fungal plant pathogens, *Zymoseptoria tricii* (Ascomycota, class Dothideomycetes,
678 Quadvlieg et al., 2011) and a *Fusarium* sp. (Ascomycota, class Sordariomycetes, Lombard et
679 al., 2015) under NT compared to ST (clusters 3 and 4 in Figure 2B). In contrast, other
680 organisms in the class Dothideomycetes relatively increased under NT including *Spegazzinia*
681 *radermacherae,* a saprotroph living on fallen seed pods (Jayasiri et al., 2019) and
682 *Sclerostagonospora*, a pathogen or saprotroph of various monocotyledons and dicotyledons
683 (Phookamsak et al., 2014) as well as other saprotrophs including *Keissleriella* (*cirsii*) and
684 *Clarireedia bennettii* (class Leotiomycetes), the latter known as pathogen of C3 turfgrasses
685 (Salgado-Salazar et al., 2018). The increase of some fungal plant saprotrophs and seed/pollen
686 attacking taxa under NT might have resulted from an increased frequency of co-occurring
687 weeds, weed richness, and weed seed bank density observed in soil under reduced tillage
688 (MT and NT) (Santín-Montanyá et al., 2020, 2018, 2016). The increase of herbivore nematodes
689 under NT (Table 2) supports this assumption.

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690 On the flipside, the most notable fungal plant pathogen antagonist strongly increased under
691 NT was a *Coprinellus curtus* (Cluster 5, Figure 2, Figure 64). As a saprotroph, *C. curtus* could
692 be expected to grow well at higher OM levels under NT. In addition, a relevant agricultural
693 isolate of *C. curtus* showed antagonistic behavior against several plant pathogens including
694 *Fusarium oxysporum* and *Rhizoctonia solani* (Nakasaki et al., 2007), which might explain the
695 observed decrease of *Fusarium* sp. in the present study. A multifunctionality of other OM-
696 degrading Basidiomycetes that relatively increased under NT, such as *Psathyrella*, *Crepidotus*,
697 and *Conocybe,* may be their plant pathogen suppressive effect, either by direct interference or
698 by indirect competition. It is speculated here that higher OM levels under NT may allow these
699 fungi to thrive.

700 4.3 Impaired wheat growth and nutritional quality under reduced tillage despite 701 enhanced soil biological indicators

702 Wheat growth, average wheat yields, and the nutritional quality indicators grain N content and
703 grain vitamin thiamine (B1) and riboflavin (B2) levels, were highest under ST and lowest under
704 NT (Table 5). Our findings are consistent with the meta-analysis by Pittelkow et al. (2015), who
705 reported increased wheat yield and N uptake under more intensive tillage practices. As N is a
706 crucial element in the synthesis of proteins and enzymes involved in the production of B
707 vitamins, it was expected that vitamin B levels would show similar patterns, directly correlating
708 with grain N content. In addition, studies have demonstrated that B vitamins are differentially
709 affected by genotype and environmental factors such as temperature, precipitation, and soil
710 conditions (Shewry et al., 2011; Batifoulier et al., 2006). These factors may further explain the
711 observed differences in B vitamin levels induced by tillage practices. In addition, this is the first
712 study reporting about tillage-induced changes in wheat technological properties as reflected
713 by a shift in A- and B-type starch distribution and on average higher content of Gliadins and
714 Glutenins under ST. As recently reviewed by Guo et al. (2023) the distribution of A- and B-type
715 starch are important technological quality indicators as they strongly influence the quality of
716 dough and final products.

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717 Explanations for wheat performance may trace to root system architecture, here measured at
718 the flowering stage. The roots data (Table 4) showed that the root-to-sprout ratio was highest
719 under NT, indicating resource allocation for soil penetration and root thickening in wheat in
720 compacted soils (Munoz-Romero et al., 2010, Watt et al., 2005, Pandey et al., 2021). Indeed,
721 wheat roots under NT had the significantly highest median root diameter, and on average
722 higher root average diameter, root maximum diameter, and larger root diameter, which were
723 consistent with descriptions of soil compaction effects on wheat (Munoz-Romero et al., 2010,
724 Atwell, 1989, Watt et al., 2005). The better performance of wheat under ST is reflected by the
725 characteristics of its root system with an increase in root area, root volume and the number of
726 root tips (Table 4). These characteristics, in turn, are reported to be linked to higher N
727 mineralization potential through: 1) root exudation associated with number of actively growing
728 root tips, considered as hot spots of microbial abundance and activity in the rhizosphere, 2)
729 root surface or length being indicative of the potential for nutrient exploration and establishment
730 of biological associations, and 3) proportion of fine roots influencing the water uptake capacity
731 and the total root surface (Freschet et al., 2021, Delaplace et al., 2015, Kuzyakov and
732 Blagodatskaya, 2015, Watt et al., 2009, Watt et al., 2008, McCully, 1999). Roots influence
733 microbes and their nutrient mobilization potential by supplying energy in what is summarized
734 as the “Rhizosphere effect” (Kuzyakov and Blagodatskaya, 2015, Nguyen, 2009, Hodge et al.,
735 2000). This could explain the significantly higher proportion of rhizosphere-associated or
736 potentially plant growth promoting taxa under ST of *Bacillus* and *Cohnella* (Firmicutes), and
737 Actinomycetes such as *Streptomyces* and *Luedemanella.*

738 Wheat grown under MT did not perform as well in regards to grain yield and quality as wheat
739 grown under ST (Table 5). In the former, root traits were characterized by on average highest
740 values for root branch points, branching frequency, and lowest root angle orientation with
741 higher frequency of shallow angled roots (Table 4). Considering that MT only loosens the soil
742 to a depth of about 15 cm, could mean easier soil penetration during the early growth stages
743 of wheat, and negative effects during later growth stages associated with a soil compaction
744 layer. At the same time, the root angle influences the vertical distribution of the roots (and their

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745 water uptake potential in deeper soil layers), which is influenced by soil compaction and could
746 affect later wheat growth (Freschet et al., 2021, Delaplace et al., 2015, Watt et al., 2009, Watt
747 et al., 2008, Watt et al., 2005). Sampling throughout the growing season as done in Munoz-
748 Romero et al., (2010) for the comparison of NT versus ST could clarify whether the
749 intermediate growth of wheat under MT is due to early growth promotion by easy soil
750 penetration and later growth inhibition by soil compaction. In addition, legacy effects of long-
751 term NT/MT on root characteristics might differ to conditions with recent/alternating tillage
752 management practices.

753 Despite higher concentration of available soil nutrients (Table 1), increased abundances of
754 prokaryotes, eukaryotes, nematodes and acari (Table 2), substrate mineralization potential
755 and enzyme cycling activity under NT (i.e. N cycling, Table 3), wheat performance was lower
756 than under ST (Table 4, Table 5). This is striking as the action of microbial grazers as well as
757 the diurnal cycles of water uptake in the rhizosphere should liberate nutrients from the microbial
758 pool for plant uptake (Snapp and Drinkwater, 2007, Clarholm, 1985) enhancing nutrient uptake
759 under NT. Thus, other factors such as nutrient competition by higher weed pressure, microbial
760 immobilization and/or slower SOM mineralization resulting in a reduced N availability or uptake
761 might have limited wheat growth and grain N content under reduced tillage (Soane et al. 2012,
762 Cooper et al. 2016, Hofmeijer et al. 2019, Kuzyakov and Blagodatskaya, 2015, Nguyen, 2009,
763 Hodge et al., 2000). Here we have outlined possible physical and biological reasons for the
764 decrease in wheat performance under reduced soil tillage intensity, however, the exact causes
765 require further research.

766 5. Conclusion

767 Our study highlights the important role of tillage in shaping soil biodiversity and functionality.
768 Varying tillage intensity is reflected in distinct shifts in both the structural and functional
769 communities within the soil. Our findings provide evidence that the formation of biocrusts under
770 NT may contribute to the accumulation of OM in upper soil layers. The lower growth
771 performance and nutritional quality of wheat grains, despite enhanced soil chemistry and

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772 biology, points to underlying root structure problems that might have limited wheat growth and
773 nutrient uptake. Addressing these issues may require the application of additional measures
774 such as the cultivation of deep rooting inter-crops or complementary tillage practices to
775 improve wheat production under no-tillage conditions. Alternatively, research into wheat
776 varieties with root systems adapted to no-tillage conditions may offer promising solutions. In
777 addition, the study showed that tillage intensity has the potential to alter technological grain
778 properties and should be considered in the subsequent use of the wheat for production.

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790 Declaration of competing interest

791 The authors declare that they have no known competing financial interests or personal
792 relationships that could have appeared to influence the work reported in this paper.

793 Data availability

794 The microbiome data presented in the study are deposited in the NCBI Sequence Read
795 Archive (SRA) repository, accession number xxx. All other data are available on Zenodo (link
796 will be added during revision).

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1104 # Tables

1105 **Table 1:** Soil chemical properties standard tillage (ST), minimum tillage (MT) and no-tillage
1106 (NT) systems, in order of decreasing tillage intensity, collected from 0-10 cm, 0-20 cm, or
1107 individually from 0-10 cm and 10-20 cm as indicated. Extraction matrices used are abbreviated
1108 as follows: Calcium Chloride ($CaCl_2$), Calcium-Acetate-Lactate (CAL), nitric acid and
1109 hydrochloric acid (AR, aqua regia). Values presented are estimated marginal means with 95%
1110 confidence intervals given in brackets. Model outputs are indicated with $p \leq 0.05$ *, 0.01 **,
1111 0.001 ***. Statistical analyses that failed ANOVA assumptions are reported in brackets.
1112 Different letters indicate significant differences between groups (α=0.05).

1113

Soil property	Depth	Tillage	ST	MT	NT
Moisture content (%)	0-20 cm	$F_{2,6}$ = 8.7 *	3.2 [3.0, 3.5] a	3.1 [2.9, 3.4] a	2.6 [2.4, 2.9] b
Soil pH_{CaCl2}	0-20 cm	$F_{2,6}$= 0.3	7.4 [7.0, 7.7]	7.3 [7.0, 7.6]	7.2 [6.9, 7.5]
Total C (%)	0-10 cm	($F_{2,8}$= 9.6 **)	0.77	0.80	1.32
Total C (%)	10-20 cm	$F_{2,8}$= 10.0 **	0.73 [0.65, 0.84] b	0.79 [0.70, 0.95] b	1.24 [0.94, 1.34] a
Total N (%)	0-10 cm	$F_{2,8}$= 29.1 ***	0.06 [0.05, 0.08] b	0.07 [0.06, 0.08] b	0.12 [0.11, 0.13] a
Total N (%)	10-20 cm	$F_{2,8}$= 16.2 **	0.06 [0.04, 0.07] b	0.07 [0.05, 0.08] b	0.11 [0.09, 0.12] a
C:N ratio	0-10 cm	($F_{2,8}$= 0.6)	12.1	10.9	11.4
C:N ratio	10-20 cm	($F_{2,8}$= 0.2)	12.2	11.3	11.7
P_{CAL} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 4.1	692 [499, 884]	677 [484, 869]	958 [766, 1151]
P_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 68.1 ***	324 [310, 340] b	335 [319, 352] b	451 [425, 480] a
K_{CAL} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 42.9 ***	1592 [1255, 1960] b	1497 [1168, 1857] b	3477 [3008, 3973] a
K_{AR} (mg kg^{-1})	0-20 cm	($F_{2,4}$= 0.1)	5290	4624	4963
Mg_{CaCl2} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 0.3	1602 [966, 2238]	1458 [822, 2093]	1742 [1106, 2378]
Mg_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 3.0	5688 [5061, 6315]	4848 [4221, 5475]	5501 [4874, 6128]
Ca_{AR} (mg kg^{-1})	0-20 cm	($F_{2,6}$= 0.6)	5896	4554	5822
Fe_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 2.1	18446 [16812, 20080]	16743 [15109, 18377]	16792 [15158, 18426]
Mn_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 0.3	310 [268, 352]	295 [253, 338]	292 [249, 334]
Cu_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 0.8	13 [12, 14]	12 [11, 13]	12 [11, 14]
Zn_{AR} (mg kg^{-1})	0-20 cm	$F_{2,6}$= 0.5	46 [41, 51]	43 [37, 48]	44 [39, 50]

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1115 **Table 2:** Abundances and diversity of soil bacteria, fungi, acari, and nematodes under standard
1116 tillage (ST), minimum tillage (MT) and no-tillage (NT) collected from 0-10 cm, 0-20 cm, or
1117 individually from 0-10 cm and 10-20 cm as indicated. Values presented are estimated marginal
1118 means with 95% confidence interval given in brackets. Model outputs are indicated with $p \leq$
1119 0.05 *, 0.01 **, 0.001 ***. Statistical analyses that failed ANOVA assumptions are reported in
1120 brackets. Different letters indicate significant differences between groups ($\alpha=0.05$).

	Unit	Tillage (T)	Depth (D)	T:D	ST	MT	NT
Abundance							
Prokaryotic 16S	Copies g^{-1} soil	$F_{2,8}$=7.7 *	0-10 cm		5837 [3309, 9479] b	14434 [9450, 21018] a	15418 [10184, 22294] a
Eukaryotic 18S		($F_{2,8}$=4.7 *)	0-10 cm		657	1761	2064
Ratio Eu:Pr	-	($F_{2,7}$=0.2 *) cov ns	0-10 cm		0.12	0.13	0.15
Nematodes total	Counts 100 g^{-1} soil	$F_{2,8}$=1.5	0-20 cm		1546 [698, 2394]	1930 [1081, 2778]	2448 [1600, 3296]
Bacterivores		$F_{2,8}$=1.0	0-20 cm		939 [315, 1562]	1269 [645, 1892]	1469 [846, 2092]
Fungivores		($F_{2,8}$=0.1)	0-20 cm		468	526	454
Herbivores		$F_{2,8}$=3.6	0-20 cm		95 [54, 201]	96 [54, 202]	301 [128, 1121]
Omnivores		na			-	-	-
Predators		na			8	21	32
Acari total	Counts 500 mL^{-1} soil	χ^2=15.1 ***	χ^2=0.0	χ^2=1.2	86 [49, 151] a	25 [14, 46] b	123 [70, 216] a
Fungivores		χ^2=14.3 ***	χ^2=0.0	χ^2=1.5	83 [47, 148] a	24 [13, 43] b	106 [60, 187] a
Predators		χ^2=13.6 ***	χ^2=0.3	χ^2=0.1	2 [1, 5] b	1 [0, 3] b	14 [6, 30] a
Herbivores		Only four observations			-	-	-
Shannon diversity							
Bacteria		$F_{2,13}$ = 11.1 **	$F_{1,9}$ = 4.3	$F_{2,9}$ = 0.9	5.97 [5.91, 6.04] a	5.98 [5.91, 6.04] a	5.80 [5.73, 5.86] b
Fungi		$F_{2,17}$ = 1.5	$F_{1,9}$ = 0.0	$F_{2,9}$ = 1.8	3.94 [3.47, 4.42]	4.49 [4.02, 4.96]	4.10 [3.63, 4.58]
Nematodes		$F_{2,8}$ = 1.0	0-20 cm		1.41 [1.22, 1.68]	1.39 [1.21, 1.65]	1.59 [1.35, 1.68]
Acari		$F_{2,16}$ = 0.2	$F_{1,9}$ = 0.0	$F_{2,9}$ = 2.1	1.34 [1.01, 1.67]	1.38 [1.04, 1.71]	1.78 [1.44, 2.11]

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1122 **Table 3:** Soil functionality assessed under standard tillage (ST), minimum tillage (MT) and no-
1123 tillage (NT) analyzed in 0-20 cm depth or individually in 0-10 cm and 10-20 cm. Soil
1124 functionality consists of indices for community level physiological profile (CLPP with basal
1125 respiration and cumulated multiple substrate induced respiration (MSIR)), extracellular
1126 enzymatic activity (EEA) involved in carbon (C, beta-glucosidase, cello-biohydrolase,
1127 xylosidase), nitrogen (N, N-acetyl-glucosidase, L-aminopeptides), and phosphorous (P,
1128 alkaline and neutral phosphatases) cycling, and nematode functional indices (enrichment
1129 metabolic footprint (EF), enrichment Index (EI), and channel Index (CI)). Values reported are
1130 estimated marginal means with 95% confidence interval given in brackets. Model outputs are
1131 indicated with $p \leq 0.05$ *, 0.01 **, 0.001 ***. Different letters indicate significant differences
1132 between groups ($\alpha=0.05$).

	Tillage	Depth	Tillage:Depth	ST	MT	NT
CLPP (µg CO_2-C g^{-1} soil h^{-1})						
Basal respiration	$F_{2,9}$ = 8.9 **	$F_{1,9}$ = 1.8	$F_{2,9}$ = 3.5	0.4 [0.0, 0.8] b	0.9 [0.5, 1.2] ab	1.4 [1.0, 1.8] a
MSIR	$F_{2,10}$ = 12.6 **	$F_{1,9}$ = 1.7	$F_{2,9}$ = 1.4	5.0 [2.3, 7.7] b	8.7 [6.0, 11.4] b	13.0 [10.3, 15.7] a
SIR-MBC **(**µg C g^{-1} soil)	$F_{2,11}$ = 18.1 ***	$F_{1,9}$ = 1.1	$F_{2,9}$ = 1.4	69 [32, 106] c	129 [92, 166] b	202 [165, 239] a
Metabolic quotient, qCO_2, as basal respiration with spline MBC	χ^2=0.5	χ^2=0.5	χ^2=5.4	0.63 [0.22,1.04]	0.87 [0.71,1.02]	0.78 [0.50,1.06]
Metabolic quotient (nominal)				0.61	0.65	0.68
Extracellular enzymatic activity (nmol substrate g^{-1} soil h^{-1})						
C cycle	$F_{2,11}$ = 11.9 **	$F_{1,9}$ = 1.0	$F_{2,9}$ = 0.1	328 [87,568] b	542 [302, 783] b	1069 [828. 1309] a
N cycle	$F_{2,14}$ = 26.5 ***	$F_{1,9}$ = 0.9	$F_{2,9}$ = 0.0	132 [105, 166] c	231 [184, 291] b	450 [358, 566] a
P cycle	$F_{2,10}$ = 6.8 **	$F_{1,9}$ = 2.4	$F_{2,9}$ = 0.6	73 [32, 115] b	111 [70, 152] ab	166 [125, 207] a
Nematode functional indices (µg C kg^{-1} soil)						
EF: C mineralized by bacterial-feeding enrichment-opportunistic nematodes (µg C kg^{-1} soil)	$F_{2,8}$ = 0.7	0-20 cm		80 [47, 160]	100 [56, 216]	131 [69, 314]
EI: Organic matter (OM) decomposition mediated by bacterial channel	$F_{2,8}$ = 0.4	0-20 cm		48 [25, 71]	47 [24, 70]	58 [35, 81]
CI: OM decomposition mediated by fungal channel	$F_{2,8}$ = 0.4	0-20 cm		50 [19, 81]	43 [12, 74]	34 [3, 65]

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1134 **Table 4:** Wheat growth performance at flowering stage in standard tillage (ST), minimum tillage
1135 (MT) and no-tillage (NT) systems. The aboveground parameters refer to individual plants
1136 collected from a circular area 9 cm in diameter and the corresponding root parameters from a
1137 depth of 20 cm. Values are estimated marginal with 95% confidence interval given in brackets.
1138 Model output are indicated with $p \leq 0.05$ *, 0.01 **, 0.001 ***. Statistical analyses that failed
1139 ANOVA assumptions are reported in brackets. Different letters indicate significant differences
1140 between groups (α=0.05).

Wheat growth parameter	Tillage	ST	MT	NT
Plant biomass (per cylinder area)				
Aboveground biomass (g)	($F_{2,6}$ = 5.1 *)	27	15	11
Head (spike) biomass (g)	$F_{2,6}$ = 3.2	9.0 [5.4, 18.7]	5.1 [3.5, 8.2]	4.6 [3.2, 7.3]
Leaf biomass (g)	$F_{2,6}$ = 6.4 *	4.7 [2.8, 7.8] a	2.6 [1.5, 4.3] ab	1.6 [1.0, 2.7] b
Stem biomass (g)	($F_{2,6}$ = 6.5 *)	13.7	7.6	4.9
Root biomass (g)	$F_{2,6}$ = 0.3	0.86 [0.50, 1.21]	0.70 [0.35, 1.06]	0.80 [0.44, 1.15]
Root to shoot ratio	$F_{2,6}$ = 5.9 *	0.03 [0.01, 0.05] b	0.04 [0.02, 0.06] b	0.07 [0.05, 0.09] a
Root system characteristics (per cylinder volume)				
Root tips (counts)	$F_{2,6}$ = 0.1	51928 [20091, 123600]	42006 [15908, 101778]	44599 [16994, 107515]
Root branch points (counts)	($F_{2,6}$ = 0.9)	70995	79941	56659
Root branching frequency (freq. mm^{-1})	($F_{2,6}$ = 1.5)	1.10	1.18	1.00
Root network area (mm^2)	$F_{2,6}$ = 0.0	12906 [8345, 17466]	12792 [8231, 17352]	12247 [7686, 16808]
Total root volume (mm^3)	$F_{2,6}$ = 0.1	12272 [6303, 18240]	10814 [4845, 16783]	10530 [4561, 16499]
Total root surface area (mm^2)	$F_{2,6}$ = 0.1	69525 [42125, 96925]	67592 [40192, 94992]	62697 [35298, 90097]
Total root length (mm)	($F_{2,6}$ = 0.6)	65102	71601	56486
Root average diameter (mm)	($F_{2,6}$ = 11.5 **)	0.34	0.29	0.35
Root median diameter (mm)	$F_{2,6}$ = 12.3 **	0.22 [0.21, 0.24] b	0.20 [0.18, 0.22] b	0.25 [0.24, 0.27] a
Root maximum diameter (mm)	$F_{2,6}$ = 0.8	3.40 [2.74, 3.98]	3.42 [2.76, 4.00]	3.78 [3.17, 4.33]
Root length diameter range <0.1 mm (mm)	$F_{2,6}$ = 1.2	9114 [4922, 18404]	11331 [5964, 23665]	6401 [3597, 12281]
Root length diameter range 0.1-0.2 mm (mm)	($F_{2,6}$ = 2.1)	21762	24884	16159
Root length diameter range >0.2 mm (mm)	$F_{2,6}$ = 0.0	33207	33127	33299
Root crown analysis				
Average root orientation (°, deg.)	($F_{2,6}$ = 1.6)	46.0°	43.9°	45.2°
Shallow angle frequency 0-30° (%)	$F_{2,6}$ = 1.6	31.2 [27.1, 35.3]	35.4 [31.3, 39.5]	33.6 [29.5, 37.7]
Medium angle frequency 30-60° (%)	($F_{2,6}$ = 0.2)	32.6	32.3	31.9
Steep angle frequency 60-90° (%)	($F_{2,6}$ = 1.5)	35.1	32.0	34.2

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1141 **Table 5**: Wheat grain yield, nutritional and technological quality in standard tillage (ST),
1142 minimum tillage (MT) and no-tillage (NT) systems. Values presented are estimated marginal
1143 means with 95% confidence interval given in brackets. Model output are indicated with $p \leq$
1144 0.05 *, 0.01 **, 0.001 ***. Statistical analyses that failed DHARMa diagnostics are reported in
1145 brackets. Different letters indicate significant differences between groups (α=0.05).

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	Tillage	ST	MT	NT
Harvest parameters				
Grain yield (dt ha^{-1})	$F_{2,6}$ = 0.8	7.3 [4.8, 9.7]	5.5 [3.0, 7.9]	6.0 [3.6, 8.5]
Thousand kernel weight (g)	$F_{2,6}$ = 1.8	17.1 [15.1, 19.1]	17.7 [15.7, 19.7]	19.3 [17.3, 21.3]
Nutrients				
C (g kg^{-1})	$F_{2,6}$ = 2.0	430 [427, 433]	429 [426, 432]	427 [424, 430]
N (g kg^{-1})	$F_{2,6}$ = 5.6 *	31 [28, 34] a	28 [25, 31] ab	25 [22, 28] b
S (g kg^{-1})	$F_{2,6}$ = 0.8	1.8 [1.7, 2.0]	1.7 [1.5, 1.9]	1.7 [1.6, 1.9]
P (g kg^{-1})	($F_{2,6}$ = 0.8)	4.06	3.88	3.92
K (g kg^{-1})	($F_{2,6}$ = 4.9)	5.43	5.72	5.19
Mg (g kg^{-1})	$F_{2,6}$ = 1.2	1.44 [1.33, 1.55]	1.40 [1.29, 1.51]	1.34 [1.23, 1.45]
Ca (mg kg^{-1})	($F_{2,6}$ = 12.9 **)	493	923	410
Cl (mg kg^{-1})	$F_{2,6}$ = 4.4	724 [613, 835]	906 [795, 1017]	768 [658, 879]
Si (mg kg^{-1})	$F_{2,6}$ = 6.7 *	191 [53, 329] b	409 [271, 547] a	131 [-7, 268] b
Fe (mg kg^{-1})	($F_{2,6}$ = 2.6)	37	30	25
Mn (mg kg^{-1})	($F_{2,6}$ = 3.6)	40	39	34
Zn (mg kg^{-1})	$F_{2,6}$ = 0.5	30 [25, 38]	28 [24, 35]	27 [23, 33]
Cu (mg kg^{-1})	$F_{2,6}$ = 0.8	5.7 [5.1, 6.6]	5.3 [4.7, 6.0]	5.3 [4.7, 6.0]
Ni (mg kg^{-1})	$F_{2,6}$ = 1.3	0.5 [0.4, 1.0]	0.4 [0.3, 0.6]	0.5 [0.3, 0.9]
Mo (mg kg^{-1})	$F_{2,6}$ = 0.9	0.2 [0.0, 0.3]	0.1 [0.0, 0.3]	0.2 [0.1, 0.4]
Co (mg kg^{-1})	$F_{2,6}$ = 1.0	0.03 [0.02, 0.05]	0.03 [0.01, 0.04]	0.04 [0.03, 0.06]
B vitamins				
Thiamine (B1) (µg 100 g^{-1})	$F_{2,6}$ = 12.5 **	6.4 [5.6, 7.6] a	4.7 [4.2, 5.3] b	4.5 [4.1, 5.1] b
Riboflavin (B2) (µg 100 g^{-1})	$F_{2,6}$ = 6.3 *	1.1 [0.9, 1.3] a	0.8 [0.6, 1.0] b	0.7 [0.5, 0.9] b
Niacine (B3) (µg 100 g^{-1})	($F_{2,6}$ = 2.3)	14.6	12.1	11.3
Pantothenic acid (B5) (µg 100 g^{-1})	($F_{2,6}$ = 5.5 *)	9.1	7.6	6.3
Pyridoxine (B6) (µg 100 g^{-1})	($F_{2,6}$ = 6.9 *)	2.9	2.2	2.1
Folate (B9) (µg 100 g^{-1})	$F_{2,6}$ = 4.6	0.6 [0.5, 0.7]	0.4 [0.3, 0.5]	0.4 [0.3, 0.5]
Technological properties				
A-type starch (>10 µm) (%)	$F_{2,6}$ = 11.2 **	93.0 [91.5, 94.5] a	91.3 [89.9, 92.8] a	89.0 [87.5, 90.5] b
B-type starch (2-10 µm) (%)	($F_{2,6}$ = 11.6 **)	6.9	8.6	10.9
C-type starch (< 2 µm) (%)	$F_{2,6}$ = 0.4	0.0 [0.0, 0.1]	0.0 [0.0, 0.1]	0.1 [0.0, 0.1] b
Glutenins (%)	$F_{2,6}$ = 3.7	1.38 [1.19, 1.56]	1.14 [0.96, 1.33]	1.11 [0.92, 1.29]
Gliadins (%)	$F_{2,6}$ = 4.0	0.67 [0.55, 0.80]	0.63 [0.50, 0.76]	0.47 [0.35, 0.60]

1146