Short-term effects of subsoil management by strip-wise loosening and incorporation of organic material

Sara L. Bauke[1,*], Sabine J. Seidel[2], Miriam Athmann[3,4], Anne E. Berns[5], Melanie Braun[1], Martina I. Gocke[1], Julien Guigue[6], Timo Kautz[7], Ingrid Kögel-Knabner[6,8], Juliette Ohan[9], Matthias Rillig[10], Michael Schloter[6,9], Oliver Schmittmann[11], Stefanie Schulz[9], David Uhlig[5,12], Andrea Schnepf[5], Wulf Amelung[1,5]

[1]Institute of Crop Science and Resource Conservation (INRES) – Soil Science and Soil Ecology, University of Bonn, Nussallee 13, 53115 Bonn, Germany

[2]Institute of Crop Science and Resource Conservation (INRES) – Crop Science, University of Bonn, Katzenburgweg 5, 53115, Bonn, Germany

[3]Institute of Crop Science and Resource Conservation (INRES) – Agroecology and Organic Farming, University of Bonn, Auf dem Hügel 6, 53121 Bonn, Germany

[4]Organic Farming and Cropping Systems, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

[5]Institute for Bio- and Geosciences – IBG-3: Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

[6]Soil Science, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Emil-Ramann-Straße 2, 85354, Freising, Germany

[7]Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt Universität zu Berlin, Albrecht-Thaer- Weg 5, 14195, Berlin, Germany

[8]Institute for Advanced Study, Technical University of Munich, Lichtenbergstraße 2a, 85748 Garching, Germany

[9]Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[10]Institute of Biology, Plant Ecology, Freie Universität Berlin, Altensteinstraße 6, 14195, Berlin, Germany

[11]Institute of Agricultural Engineering, University of Bonn, Nussallee 5, 53115 Bonn, Germany

[12]Institut für Geologische Wissenschaften, Freie Universität Berlin, 12249 Berlin, Germany

*corresponding author: sarabauke@uni-bonn.de

ABSTRACT

Agricultural production in Central Europe increasingly suffers from extreme drought events. Improving root access to nutrient and water resources in the subsoil below the plow layer is a potential option to maintain productivity during dry summers. Here, we tested a strip-wise subsoil amelioration method that combines subsoil loosening with organic matter incorporation into the subsoil (biowaste or green waste compost) and compared it with a treatment of only subsoil loosening and a non-ameliorated control. A field experiment with randomized block design was conducted on a Luvisol with an argic horizon (Bt), with a rotation of spring barley and winter wheat. In the first two years after amelioration, we monitored soil physico-chemical parameters, microbial biomass, and shoot and root growth at anthesis as well as harvested grain yield and quality. Subsoil loosening with organic matter incorporation significantly decreased soil bulk density at the depth of compost incorporation when biowaste compost was used, but not when green waste compost had been incorporated. Nutrient stocks, nutrient availability and microbial biomass were not consistently affected by the subsoil amelioration. Nevertheless, the incorporation of organic material, especially biowaste compost, significantly increased root growth into the subsoil and subsequently significantly enhanced crop nutrient uptake, biomass and grain yield production. Green waste compost incorporation had less pronounced effects, with an increase in grain yield only in the second year after amelioration. Differences in crop development could not be explained by any single soil parameter, suggesting that it was rather a combined effect of loosened subsoil and better supply of subsoil resources that resulted in an increase in subsoil root length density and subsequently led to better crop performance.

KEYWORDS

Subsoil management; microbial biomass; soil nutrients; stable isotopes; root growth; grain yield

1. INTRODUCTION

With a growing global population and concomitant decline in available agricultural land area due to urbanization and land degradation (Kopittke et al., 2019; Webb et al., 2017), securing agricultural food production remains an urgent challenge. Available soil resources are additionally strained by a changing climate, which is projected to cause longer and more intense summer droughts in Central Europe (Markonis et al., 2021; Pfeifer et al., 2015), resulting in severe limitations of crop development during the critical stages of anthesis and grain yield formation (Lüttger and Feitke, 2018).

Large quantities of water and nutrients that may help to sustain crop growth during such periods of drought are located in the subsoil, beneath the tilled topsoil horizons (Gocke et al., 2021; Kautz et al., 2013; Wiesmeier et al., 2013). As most crops have root systems that can extend down to a depth of one meter and deeper (Canadell et al., 1996; Fan et al., 2017), resources in the subsoil are potentially accessible to crops. Studies in which root growth into the subsoil was facilitated, e.g., by the presence of biopores, showed that water and nutrient resources in the subsoil can contribute substantially to plant nutrition, especially during dry spells (Gaiser et al., 2012; Seidel et al., 2019; Thorup-Kristensen et al., 2020). The accessibility of these subsoil resources can be limited by the chemical properties of the subsoil, such as high acidity or alkalinity, or by physical properties such as poor structure or high soil density, e.g. due to high clay content (de Oliveira and Bell, 2022; Sale et al., 2021). For Germany in particular, a nation-wide survey revealed that root growth into the subsoil is limited by dense root restricting layers on 71% of all agricultural land (Schneider and Don, 2019). While most of these root restricting layers have a pedogenic or geogenic origin, at least 13% of soil compaction is assumed to result from agricultural management practices, occurring mainly in the upper subsoil, i.e., between 30 and 50 cm soil depth (Schneider and Don, 2019).

Attempts to overcome compacted and pedogenically dense subsoil layers include deep loosening of the soil without intensive mixing of topsoil and subsoil, soil flipping or inversion where topsoil material is incorporated into the subsoil (Schneider et al., 2017), or subsoil manuring with loosening and incorporation of mineral or organic fertilizers into the subsoil (Gill et al., 2009; Ma et al., 2009; Sale et

al., 2019). Deep loosening of the soil often results in an initial increase in yields, but re-compaction may occur (e.g., Larney and Fortune, 1986) causing even higher soil density after a few years than before deep loosening if agricultural management is not adapted, especially in silty soils. This re-compaction is likely due to the degradation of the initial soil structure (Schneider et al., 2017). In contrast, after soil flipping and incorporation of topsoil material (i.e., material typically enriched with fertilizer and organic matter) into the subsoil, organic matter was even preserved for several decades in the subsoil (Alcantára et al., 2016; Schiedung et al., 2019). This placement of organic matter-rich material into the subsoil may help to preserve the loosening effect in the subsoil by stabilizing soil structure (Jayawardane et al., 1995), as well as to lower bulk density and penetration resistance (Getahun et al., 2018). Deep loosening, however, can also dilute nutrient supply in the topsoil by admixing of subsoil material with relatively lower nutrient concentration, which may limit productivity in the following years. Accordingly, some studies on soil flipping or subsoil manuring reported increased yields compared to conventional management or even topsoil applications of organic matter (Getahun et al., 2018; Gill e al., 2009; Sale et al., 2019; Schneider et al., 2017), while others did not (Jin et al., 2023; McPhee et al. 2023).

Even with increased yields, both deep loosening and soil flipping are frequently not economically viable when applied uniformly across a field, and many farmers are skeptical towards such labor-intensive and highly destructive measures (Frelih-Larsen et al., 2018). Especially soil flipping, where subsoil is brought to the soil surface, can even initially decrease soil fertility and, if subsoils are rich in clay, also reduce the workability and trafficability of the field instead of creating positive effects for crop growth (Schneider et al., 2017). Therefore, we suggest that an amelioration system of subsoil loosening with simultaneous incorporation of organic matter into the loosened subsoil is a more viable management strategy when it is carried out in interspaced furrows (e.g. at one meter distance from one another), and when topsoil and subsoil materials are not mixed or turned. The technical basis for such a system that avoids mixing of topsoil and subsoil by removing the topsoil before and placing it back after the subsoil loosening run has recently been introduced. The technical feasibility of this system was illustrated by Jakobs et al. (2017). First results on changes in soil moisture, soil mineral nitrogen (N) contents, and

crop yields after subsoil amelioration were presented in Jakobs et al. (2019) and Schmittmann et al. (2021), but a holistic assessment of crop performance and its drivers is missing.

Assessing the sustainability of such subsoil amelioration measures should go beyond a purely quantitative assessment of yield and grain yield quality, and should additionally evaluate water and nutrient uptake of crops. The ability of crops to utilize water and nutrients from the subsoil primarily depends on the rooting density and depth and can additionally be enhanced via soil microbiota contributing to nutrient mobilization and organic matter decomposition from the subsoil (Gregory, 2006; Uksa et al., 2015), especially after subsoil disruption (Salomé et al., 2010). As such, the incorporation of organic matter into the subsoil has been shown to increase the amount of available water (Getahun et al., 2018; Jakobs et al., 2019), the contents of mineral N in the subsoil (Jakobs et al., 2019), and crop nutrient uptake as recently shown for magnesium (Mg) using $\delta^{26}Mg$ values or for mineral nutrients in general when using the strontium ratio ($^{87}Sr/^{86}Sr$) as a proxy for identification of nutrient uptake depth (Uhlig et al., 2022, 2023).

Here, we evaluated a subsoil management strategy with and without compost injection into subsoil within a 2-year field experiment, involving a rotation of spring barley and winter wheat. We hypothesized that i) strip-wise mechanical loosening of the subsoil only exhibits annual, short-term effects on soil physical properties (i.e., in the first year) but no effect on soil chemical properties, thus providing only limited benefits for crop production. Further, we hypothesize that strip-wise mechanical loosening with incorporation of organic matter will ii) change soil physico-chemical and microbial parameters towards an increased availability of nutrients, and will iii) enhance root growth, nutrient uptake and plant development during the growing season, thereby iv) ultimately increasing yield quantity and grain quality. To test these hypotheses, we analyzed soil, microbial and plant parameters during anthesis and harvest periods of the first two years after strip-wise subsoil amelioration in a field experiment in Germany on a Haplic Luvisol with clay accumulation (Bt horizon) in the subsoil.

2. MATERIALS AND METHODS

2.1 Experimental site and subsoil management

The field experiment was established at the Klein-Altendorf experimental station of the University of Bonn, located at Rheinbach near Bonn, Germany (50° 37' 21" N, 6° 59' 29" E). The soil at the study site was classified as Haplic Luvisol (Hypereutric, Siltic) and is characterized by a silty clay loam texture with clay accumulation in the argic horizon (Bt) between 45 and 95 cm soil depth. For more detailed analyses of soil texture and chemical properties of a soil profile in Klein-Altendorf near the current field trial see Barej et al. (2014). The climate at the experimental station can be described as temperate humid with maritime influence. The mean annual air temperature and precipitation (1991 to 2020) are 10.3°C and 733 mm.

The field experiment has a total size of 1.5 hectare and was subdivided into three experimental subtrials (CF1-1; CF1-2; CF1-3), which started in three consecutive years (2016 to 2018, Figure S1, Supplementary Material). Each subtrial was divided into 24 plots; individual plot size was 15 x 3 m (45 m^2) in CF1-1 and 20 x 3 m (60 m^2) in CF1-2 and CF1-3. Treatment and control plots were arranged in a randomized block design with three field replicates. The specific treatments within each trial varied slightly depending on the experimental question. For this study, we selected three treatments that were implemented consistently across all subtrials: Deep loosening without organic matter addition (DL), deep loosening with incorporation of biowaste compost (DLB) and deep loosening with incorporation of green waste compost (DLG; existing only in CF1-2). These treatments were compared to a control without subsoil management.

Before the start of the experiment lime was applied uniformly across the field with 3 t ha^{-1} (2015) and 4 t ha^{-1} (2016) as converter lime (Lhoist Germany, Rheinkalk GmbH). At the start of each experimental subtrial, subsoil management with or without organic matter addition was carried out along one 30 cm wide strip centrally within the plot of 3 m width and along the full plot length of 15 or 20 m (Schmittmann et al., 2021). A detailed description of the subsoil management is given in Jakobs et al. (2019). In brief, the topsoil (0 – 30 cm) on this furrow was removed with a plow board of 30 cm width. Subsequently, the subsoil within this 30 cm wide furrow was loosened from 30 to 60 cm depth with a

single deep tine. In treatments with organic matter addition, the loosened subsoil was additionally mixed with either biowaste compost or green waste compost. Finally, the loosened subsoil was moderately recompacted by a depth wheel, then the topsoil was placed back with a board. Subsoil management was carried out in autumn (September) of the respective start year (2016 in CF1-1, 2017 in CF1-2 and 2018 in CF1-3) when the soil was dry, to minimize the risk of management-induced soil compaction. After subsoil management, regular seedbed preparation was carried out with a chisel plow and rotary harrow (15 and 10 cm working depth, respectively). Mustard (*Sinapis alba* L.) was first sown as a cover crop, to allow for soil rest and to minimize any negative effects of soil movement during the amelioration procedure. The cover crop was then mulched in March of the following year, followed by a rotation of spring barley (*Hordeum vulgare* L.) and winter wheat (*Triticum aestivum* L.). An overview of the timeline, cultivars, management during the experiment and sampling dates is given in Figure S1 and Table S1 (Supplementary Material).

Both compost types were obtained from a nearby composting plant (Kompostwerke Rhein Sieg, Swistal, Germany). The biowaste compost was a finished rotten compost of kitchen and garden wastes collected from private households by the municipality. The green waste compost was from tree and shrub cuttings from public gardens and parks. The green waste compost had a dry matter content of 60.7% and C and N contents in dry matter of respectively 29.3% and 1.1%, while biowaste composte had a dry matter content of 66.8% with 25.6 % C and 1.9% N in dry matter. Further details on particle size and element composition of the composts are given in Table S2. Both biowaste and green waste compost were added to the 30 cm wide furrow as 5 kg m^{-1} of compost fresh mass with dry matter proportions given above. This amount is equal to 50 t ha^{-1} for the proposed management system where all added amendments are concentrated in the furrow interspaced at 1 m distance across the field and no material is added to the 70 cm of soil in between the furrows.

The results presented in this paper focus on monitoring of soil, microbial, root and shoot parameters directly within the ameliorated area as contrasted to a control without amelioration for the main crops spring barley and winter wheat in the first and second year after subsoil management. Soil and plant samples were collected around anthesis from CF1-2 in 2018 and CF1-3 in 2019 (first year after

amelioration) and CF1-1 in 2018 and CF 1-2 in 2019 (second year after amelioration; for specific dates see Table S1, Supplementary Material). Note that grain and straw yield, grain quality parameters, root growth, soil bulk density and available soil mineral nitrogen (N_{min}) data for one year of one of the experimental subtrials (CF1-1 in 2018) were previously reported in Jakobs et al. (2019) and are reused here. Also, the Mg isotope composition of ears was previously reported in Uhlig et al. (2022) and Uhlig (2022) and reused in the present paper.

2.2 Soil sampling and analyses at anthesis

2.2.1 Sampling

At anthesis of spring barley and winter wheat in each of the field experiments and experimental years (Table S1, Supplementary Material), one soil core was taken in the middle of each plot in the subsoil amelioration furrow or in the middle of the control plot for analysis of soil bulk density, pH, and C and N contents. Cores were collected with a driving hammer probe, using a sheath probe with an inner diameter of 60 mm (Nordmeyer Geotool GmbH, Berlin, Germany). The soil core was directly wrapped in a polyethylene film liner inside the cylinder during coring. All intact soil cores were transported to the laboratory and stored at 4 °C until being cut into layers of eight depth levels (i.e., 0–15 cm, 15–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–70 cm, 70–78 cm, 78–100 cm). The depth intervals of 30 and 60 cm were chosen to represent the lower boundary of the topsoil and amelioration layer, respectively. The additional depth increments at 40, 50, 70 and 78 cm were assigned according to a pre-sampling screening of mean horizon depth across the area of the entire experimental field. A correction of core length due to compaction during drilling or stretching after extraction from the soil was applied when cutting the core into the aforementioned depth intervals (Walter et al., 2016). We assumed that the top 30 cm of the soil was unaffected by compaction or stretching so that any difference in core length was attributed to the compaction or stretching of the soil below 30 cm (Walter et al., 2016).

Additionally, in spring of each year (dates see Table S1, Supplementary Material), soil samples for analysis of N_{min} concentration were collected with a soil auger to 100 cm depth, divided into samples

of 0-30 cm, 30-50 cm, 50-60 cm, 60-70 cm and 70-100 cm depth (the depth increments of 40 and 78 cm were omitted here to reduce the number of samples for analysis). Samples were again taken at the center of the plot within the area of the furrow in the amelioration treatments. In the 0-30 cm layer, 8 samples per plot were collected and merged, in the other soil layers 4 samples per plot were collected and mixed into a composite sample, which was immediately cooled and stored frozen at -18 °C. Finally, samples for nutrient and microbial analysis were collected in the same way at anthesis. For all sampling dates, these composite samples were each split into one subset for nutrient analyses and one subset for microbial analyses. Both subsets were cooled directly after sampling, then frozen at -18 °C.

2.2.2 Physicochemical parameters

Samples from the soil cores were air-dried, weighed and sieved to ≤ 2 mm particle size, the weight of the coarse fraction > 2 mm (rocks, roots and organic matter from the composts) was recorded. It should be noted that none of the samples contained large quantities of coarse material, hence the volume of the coarse material did not affect the precision of the bulk density estimation. Soil bulk density was thus calculated as the mass of dry soil after subtraction of the mass of coarse fragments (> 2 mm) and roots (dry soil mass = total soil mass– coarse fragment mass – root mass) per total volume for each soil depth segment. The volume was calculated from the cross-sectional area of the soil corer (cm^2) and the sampling depth interval (cm). The pH and electrical conductivity (EC) were measured in a 1:5 (w:w) soil to water mixture with deionized water after 1 h of horizontal shaking followed by 1 h of immobility. A subsample of approximately 5 g of sieved soil was ball-milled and used to measure total C and N by dry combustion (HEKAtech EuroEA 3000, HEKAtech GmbH, Wegberg, Germany). All soil samples were below the detection limit for the determination of inorganic C by calcimetry with 4M HCl, so the total C contents are considered equal to organic C contents.

For determination of soil ammonium (NH_4^+) and nitrate (NO_3^-) concentration, composite samples collected by soil auger were slowly thawed, a subsample was extracted with 0.5M potassium sulfate solution and analyzed photometrically with a continuous flow analyzer (Seal QuAAtro 39, Norderstedt,

238 Germany; wavelengths 540 nm and 660 nm; VDLUFA 1991). Nitrate and NH_4^+ contents were
239 summarized as N_{min}. Gravimetric soil water content was analyzed from 50 g of soil per sample to correct
240 to N_{min} content in dry soil.

241 After removal of the subsample for N_{min} analyses and water content determination, the remaining
242 sample amount was dried at 40 °C and sieved to ≤ 2 mm particle size for subsequent analyses of plant
243 available phosphorus (P) and potassium (K). One of the standard methods for plant available P and K
244 in Germany is extraction by calcium acetate calcium lactate solution (CAL) according to Schüller
245 (1969). Briefly, a subsample of 5 g of soil was shaken with 100 mL of a buffered extraction solution
246 containing calcium lactate, calcium acetate and acetic acid (pH 3.7 - 4.1) for 2 h, then filtered over a
247 paper filter. For determination of plant-available P (P_{CAL}) contents, extracts were reacted with
248 molybdenum blue solution and ascorbic acid (Murphy and Riley, 1962), then measured on a
249 spectrophotometer (Specord 205, AnalytikJena GmbH, Jena, Germany). Plant-available K (K_{CAL})
250 contents were measured using an atomic absorption spectrometer (NovAA 400 P, AnalytikJena GmbH,
251 Jena, Germany). Samples were measured in duplicate to determine measurement error, which was < 1%
252 for P_{CAL} and mostly < 5% for K_{CAL}.

253

254 *2.2.3 Calculation of nutrient stocks*

255 As deep loosening in the DL and DLB treatment may have resulted in less soil mass and thus also lower
256 absolute nutrient amounts than in the control, soil C, N, P_{CAL} and K_{CAL} concentrations were converted
257 to stocks [kg ha^{-1}] by correction to an equivalent soil mass and multiplication with the respective depth
258 increment and bulk density of the depth increment according to von Haden et al. (2020). Equivalent soil
259 masses (ESM) were calculated using the control plots of the respective year and experimental trial as a
260 reference. Soil nutrient concentrations before ESM correction are given in Table S4 (Supplementary
261 Material).

262 Soil bulk density data for treatment DL in CF1-2 (2018) were not available. As bulk density data for all
263 other treatments in CF1-2 did not change from 2018 to 2019, we used bulk density data of the same

treatment from 2019 to calculate P_{CAL} and K_{CAL} stocks for the DL treatment. Further, as soil N_{min} content is usually highly dynamic throughout the season, soil N_{min} contents were not converted to stocks.

2.2.4 Microbial parameters

The frozen subsamples of each composite sample were freeze-dried for 48 h and homogenized using a vortexer. DNA was extracted from 200 mg of each sample using a protocol designed for subsoil DNA extraction (Guerra et al., 2020). This DNA was used for real-time quantitative PCR (qPCR) analysis to quantify bacterial, archaeal and fungal biomass. SYBR Green® assays were performed on a 7300 real-time PCR machine (Thermo Fisher Scientific, Germering, Germany). Each assay contained 12.5 µl SYBR Green® (Therma Fisher Scientific), 5 pmol forward and reverse primer (Metabion, Germany), 0.5 µl 3% BSA (Sigma Aldrich, Deisenhofen, Germany) and 11 µl DEPC-treated water. Primers, thermal profiles and the source of calibration standards are summarized in the Table S3 (Supplementary Material). Prior to quantification, the samples were tested for PCR inhibition by performing a dilution test. Each qPCR run contained 1/80 diluted samples, a triplicate standard series (10^8 to 10^2 gene copies μl^{-1}) and no template controls. The quality of each qPCR run was checked by melting curve analyses and electrophoresis of selected samples on a 1.5% agarose gel. The R^2 of the standard series was above 0.99 for all qPCR runs. The amplification efficiency calculated by $E=10^{(-1/slope)}-1$ was above 91% for bacteria and above 80% for fungi. Gene copy number was determined per gram of soil.

2.3 Root growth analyses

Root-length density (RLD) was quantified with the profile wall method as described by Böhm (1979) at stem elongation (BBCH stage 31-35) and anthesis (BBCH stage 61) for spring barley and at anthesis (BBCH stage 61) for winter wheat (dates see Table S1, Supplementary Material). A trench with a depth of about 130 cm (spring barley) or 230 cm (winter wheat) was installed at one end of each plot using an excavator. Afterwards, a 100 cm wide vertical profile wall was flattened transversely to the plant

289 rows with a spade and sharp blades, roots exposed from the profile wall were cut off, and 0.5 cm of soil
290 was rinsed off with tap water from a crop sprayer with 300 kPa pressure, simultaneously scratching
291 with a fork, exposing the roots present in this soil volume. A 100 x 60 cm length times width counting
292 frame with a 5x5 cm grid was placed on the profile wall. Root length was quantified by visual estimation
293 of root-length units (RLU, root pieces of 0.5 cm length uncovered by the spraying procedure) in squares
294 of 5x5 cm size in a range of 100 cm width, from surface soil until 100 cm depth (spring barley) or 200
295 cm depth (winter wheat). Roots in holes were not considered. RLU from the soil profile wall were
296 converted into root length density (RLD, cm cm^{-3}) for each 5 cm depth layer. Data were evaluated in
297 two distance classes: within the amelioration furrow, which was set to have a lateral extension of 30
298 cm, and the area adjacent to the amelioration furrow, which was set to have a lateral extension of 35 cm
299 to each side of the amelioration furrow. Depending on experimental year, subtrial and root counting
300 person, the counting frame was positioned slightly differently regarding the position of the amelioration
301 furrow, resulting in 6 internal repetitions of 5x5 cm squares (except for CF 1-2 in 2018: 3 internal
302 repetitions) per 5 cm depth level for the amelioration furrow, and between 7 and 14 internal repetitions
303 for the area adjacent to the amelioration furrow. In the control plots, all 20 squares were used for the
304 analysis. In CF 1-2 2018, at the first sampling date at EC 32-35 data were collected only from two field
305 replicates. Profile wall data was also used to determine rooting depth (equivalent to the maximum depth
306 level with roots present) and cumulative root distribution.
307

308 **2.4 Above-ground biomass sampling and analyses at anthesis**

309 *2.4.1 Leaf area index*

310 The leaf area index (LAI) was measured non-destructively with a SS1 SunScan Canopy Analysis
311 System (Delta-T Devices Ltd, Cambridge, England). Twenty SunScan measurements were conducted
312 in each plot: 10 measurements were conducted in the non-ameliorated area of the plot and 10
313 measurements above the amelioration furrow. Calibration of the SunScan data was done based on four
314 non-destructive measurements of LAI with SunScan and then destructive measurement of LAI of the

plants in the measured area with a LI-3100C Area Meter (LI-COR Biosciences GmbH, Bad Homburg, Germany).

2.4.2 Sampling

At anthesis, crop shoot material was sampled from two areas of 0.5 x 0.5 m size centrally in the plot, i.e., including the area directly above the amelioration furrow as well as adjacent plants. Shoot dry matter was determined by weighing after oven drying (105°C).

An additional set of plant samples was collected in CF1-2 in 2018 (first year after amelioration) and CF1-1 in 2018 (second year after amelioration) for isotope analyses. These samples were immediately frozen at −20°C on the day of sampling to prevent further fractionation or reallocation processes that may affect isotope ratios. For analysis, whole above ground plants were either air-dried (40 °C) for C and N isotope analyses or dried by lyophilization for a minimum of 24 h at −55 °C using a Christ Beta 1-8LD plus freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) for Fe and Mg isotope analyses. For C and N isotope analyses only the flag leaf was milled and analyzed. For Fe and Mg isotopes plant organs were dissected into bulk ear, stem and leaves and were milled in 100 ml sealable HDPE bottles equipped with tungsten carbide milling balls using a shaker (Collomix Agia 200 Viba 330, Collomix GmbH, Gaimersheim, Germany).

2.4.3 Plant nutrient content and isotope analyses

Shoot samples were ground (Retsch RS 1) and analyzed for N (dry combustion with a Eurovector EA 3000, Pavia, Italy), P (photometrical detection with a continuous flow analyzer (Seal QuAAtro 39, Norderstedt, Germany) and K concentration (atomic absorption spectrometry; Analyst 200, PerkinElmer, Waltham, USA). The nutrient concentrations were converted to shoot N, P and K stocks (kg ha^{-1}). Plant nutrient utilization efficiency (g dry mass g^{-1} nutrient) at anthesis was calculated according to Siddiqi and Glass (1981) as the amount of shoot dry biomass divided by amount of nutrient

in dry biomass for N (nitrogen utilization efficiency, NUE), P (phosphorus utilization efficiency, PUE) and K (potassium utilization efficiency, KUE).

Carbon and N isotopes were analyzed on 7 mg subsamples of the milled flag leaf sample. Samples were analyzed by thermal combustion in a pyrocube (Elementar Analysensysteme GmbH, Langenselbold, Germany) coupled to a visION isotope ratio mass spectrometer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Measurements were calibrated using the international reference substances acetanilide (Acetanilide #1, Schimmelmann Research, Indiana University), cellulose (IAEA-CH-3) and ammonium sulfate (IAEA-311). ^{13}C to ^{12}C isotope ratios were expressed in δ notation in per mill relative to the Vienna Pee Dee Belemnite standard ($\delta^{13}C_{VPDB}$); ^{15}N to ^{14}N isotope ratios were expressed as $\delta^{15}N$ in per mill relative to atmospheric nitrogen ($\delta^{15}N_{air}$).

To analyze stable isotope ratios of Mg and Fe, we followed the procedures outlined in Uhlig et al. (2022) and Wu et al. (2021). Detailed descriptions of the extraction and purification procedures are given in Text S1 in the Supplementary Material. Magnesium isotope ratios were measured by multi collector inductively coupled plasma mass spectrometry (MC-ICP-MS) on a Nu Plasma II (Nu Instruments Ltd, Wrexham, UK). Results were expressed as the per mill difference of the Mg isotope ratio of the sample relative to the ERM-AE143 using the delta notation: $\delta^{26/25}Mg=[(^{26/25}Mg/^{24}Mg)_{sample}/(^{26/25}Mg/^{24}Mg)_{ERM\text{-}AE143}-1]\times1000$. $\delta^{26/25}Mg_{ERM\text{-}AE143}$ values were converted to the DSM-3 scale using the conversion factors -3.284‰ ± 0.027‰ for $\delta^{26}Mg$ and -1.681‰ ± 0.021‰ for $\delta^{25}Mg$ (Vogl et al. 2020) and equation 2 in Young and Galy (2004). The accuracy and long-term external reproducibility were assessed by processing the bracketing standard ERM-AE143 and NIST SRM 1515 Apple leaves with the same analytical methods as field samples and reported in Uhlig et al. (2022). The long-term external reproducibility was about ±0.07‰ (2SD) for $\delta^{26}Mg$. The Fe isotope composition was also determined by MC-ICP-MS (Nu Plasma II, Nu Instruments Ltd, Wrexham, UK). The results of Fe isotope analysis in samples were expressed relative to standard IRMM-014 (as recommended in Dauphas et al., 2017) as $\delta^{57/56}Fe=[(^{57/56}Fe/^{54}Fe)_{sample}/(^{57/56}Fe/^{54}Fe)_{IRMM\text{-}014}-1]\times1000$. The accuracy and long-term external precision was assessed by processing the bracketing

366 standard IRMM-524a, i.e., the parent material of IRMM-014 with the same analytical methods as for
367 the field samples. The long-term external reproducibility was 0.11‰ for $\delta^{56}Fe$ and 0.19‰ for $\delta^{57}Fe$.

368 Both $\delta^{25}Mg$ and $\delta^{57}Fe$ were only analyzed for the purpose of quality control. These data are reported in
369 Table S9 (Supplementary Material), but were not considered further in the main text (Table 3).

370

371 **2.5 Yield and yield quality at harvest**

372 Plants were harvested manually along 1 m of plant rows, with a row distance of 12.5 cm. In each plot,
373 two 1 m rows were harvested in the center of the control plot or above the amelioration furrow in the
374 treatment plots. In 2019, plots were additionally harvested with a plot harvester along half the length of
375 a plot (10 m) and with 1 m width, comprising the amelioration furrow (30 cm) as well as 35 cm non-
376 ameliorated area to the left and right of the amelioration furrow, respectively.

377 From the manual harvests in 2018 and 2019 plant biomass was separated into grain and straw. From
378 these samples grain yield, straw dry matter and total above ground biomass dry matter (as the sum of
379 grain and straw dry matter) were determined and converted to mass per hectare area basis [kg ha^{-1}]
380 according to the standard procedures of the German Federal Plant Variety Office, which includes a
381 residual water content of 14% in the harvested grain yield (Bundessortenamt, 2000).

382 For grain quality measurements, a subsample of the grain yield was sieved through a sieve stack with
383 > 2.8 mm, 2.5-2.8 mm, 2.2-2.5 mm and <2.2 mm mesh size. In 2018, this subsample was obtained from
384 the manual harvests, while in 2019 the subsample was obtained from the plot harvests (i.e., including
385 both ameliorated and non-ameliorated area). Yield quality parameters such as grain moisture content,
386 and grain protein, starch and fiber content were determined using near-infrared technology (Perten
387 DA7250TM NIR analyzer).

388

389 **2.6 Statistical analyses**

Statistical analyses and data visualization were done in R (R Core Team, 2022; version 4.0.2).

For the soil physicochemical parameters, where only one soil core per plot was extracted, the lack of repeated (composite) sampling may include the risk of not sampling the plot in a fully representative manner, increasing data heterogeneity and potentially weakening the statistical power of the comparisons. However, nutrient analyses were done on composite samples of four to eight cores per plot, and we did not identify greater data variability in the physicochemical data compared to the nutrient concentration data. Data from all soil sampling methods were thus treated in the same way in the subsequent statistical analysis.

To facilitate presentation of the results of the separate soil sampling campaigns, data of the individual depth increments were aggregated to depth intervals of 0-30 cm, 30-60 cm and 60-100 cm. Further, the data of the individual experimental subtrials were summarized per experimental year after amelioration, i.e., data from CF1-2 2018 and CF1-3 2019 are summarized as Year 1 after subsoil amelioration and data from CF1-1 2018 and CF1-2 2019 are summarized as Year 2 after subsoil amelioration. Note that due to the experimental design (DLG treatment only included in trial CF1-2) this results in $n = 3$ for the DLG data per year after amelioration, while for all other treatments and the control $n = 6$. Data for each experimental subtrial separately can be found in the Supplementary Material (Tables S5, S6, S7, and S9).

Data were analyzed using linear mixed effect models (lmer function of the lme4 package) with treatments and year after amelioration considered as fixed effects, the specific experimental subtrial that the samples were obtained from was considered as a random effect. Significant differences ($p < 0.05$) were then determined using a Tukey Contrasts calculation (based on glht function of the multcomp package) for the treatment contrasts and for the contrasts of the two experimental years after amelioration across all treatments and control. For root data, significant differences were determined for each experimental subtrial by ANOVA followed by Tukey's HSD test ($p < 0.05$) for each soil depth.

3. RESULTS

3.1 Soil physicochemical properties

Bulk density in the ameliorated subsoil depth (30-60 cm) was significantly lower in the treatments where organic matter was incorporated into the subsoil (DLB and DLG) than in the control (Table 1).

Table 1 Soil properties of the experimental field for the control (C), and treatments deep loosening (DL), deep loosening with incorporation of biowaste compost (DLB) and deep loosening with incorporation of green waste compost (DLG). All samples were obtained from the center of the plot above the amelioration furrow. Values are given as mean ± standard error (in parentheses) per treatment and year 1 (Y1, spring barley) and year 2 (Y2, winter wheat) after subsoil amelioration, respectively. Note that due to the experimental design for C, DL and DLB n = 6 and for DLG n = 3 (see methods section). Different letters indicate significant differences ($p \leq 0.05$) among treatments across both experimental years. The p values given in the last column indicate significant difference among experimental years across all treatments and control.

	C			DL			DLB			DLG			*p value*
	Y1	**Y2**		**Y1[1]**	**Y2[2]**		**Y1**	**Y2**		**Y1**	**Y2**		***Y1-Y2***
Bulk density [g cm^{-3}]													
0-30 cm	1.4 (0.0)	1.4 (0.0)	A	1.3 (0.0)	1.3 (0.0)	A	1.3 (0.0)	1.2 (0.0)	B	1.3 (0.0)	1.2 (0.0)	B	n.s.
30-60 cm	1.5 (0.0)	1.5 (0.0)	A	1.6 (0.0)	1.4 (0.0)	AB	1.5 (0.0)	1.3 (0.0)	C	1.4 (0.0)	1.3 (0.0)	BC	< 0.05
60-100 cm	1.6 (0.0)	1.6 (0.1)	A	1.4 (0.0)	1.5 (0.0)	B	1.6 (0.0)	1.6 (0.0)	AB	1.6 (0.0)	1.6 (0.0)	AB	n.s.
pH [-]													
0-30 cm	7.7 (0.0)	7.9 (0.0)	A	7.8 (0.1)	7.8 (0.1)	AB	7.9 (0.0)	7.9 (0.0)	B	7.8 (0.0)	7.8 (0.1)	A	n.s.
30-60 cm	7.9 (0.0)	7.9 (0.1)	A	7.9 (0.1)	7.8 (0.1)	A	8.0 (0.0)	8.2 (0.0)	B	7.9 (0.0)	7.8 (0.0)	B	n.s.
60-100 cm	8.3 (0.3)	8.4 (0.3)		8.0 (0.1)	7.9 (0.1)		8.0 (0.0)	8.2 (0.1)		8.0 (0.0)	7.7 (0.0)		n.s.
Electrical conductivity [µS cm^{-1}]													
0-30 cm	56.4 (3.6)	44.9 (5.8)	A	52.2 (5.7)	44.9 (4.2)	A	76.2 (13.0)	73.4 (8.3)	B	73.8 (2.1)	63.3 (3.2)	AB	< 0.001
30-60 cm	41.3 (3.0)	32.6 (3.8)	A	31.7 (3.3)	32.7 (3.7)	A	53.6 (10.7)	70.1 (11.8)	B	50.6 (2.9)	42.0 (3.7)	AB	< 0.01
60-100 cm	55.4 (5.8)	40.3 (7.8)		38.3 (1.2)	40.3 (7.1)		47.3 (7.9)	53.1 (5.7)		48.6 (4.4)	33.3 (0.3)		< 0.001

[1] n= 3 due to missing data, [2] n= 4 due to missing data

Only loosening of the subsoil (DL treatment) did not significantly reduce bulk density, instead this treatment showed the highest of all bulk density values observed in the experiment in year 1 after subsoil amelioration (not significant, Table 1). Bulk density was also significantly reduced in the topsoil of the DLB and DLG treatments compared to the control, and in the deeper subsoil of the DL treatment (Table 1). Soil electrical conductivity and soil pH were significantly elevated in DLB and DLG treatments in the amelioration depth and also the topsoil in the DLB treatment showed significant higher pH and electrical conductivity than the control (Table 1). In the deeper subsoil (60 – 100 cm), electrical conductivity and pH were not significantly affected by the amelioration treatments. Only electrical conductivity differed significantly between year 1 and year 2 after amelioration, when considered across all treatments. Electrical conductivity in the control and DLG treatment decreased from year 1 to year 2, but increased in the DLB treatment.

After ESM correction, C and N stocks in the topsoil and at amelioration depth were larger in DLG and particularly DLB treatments than in the plots without organic matter incorporation, but this effect was significant only for the C stocks in the DLB treatment (Table 2). However, for DLB treatments in the deep subsoil below amelioration depth, C and N stocks were lower than in the control plots (not significant). Stocks of available nutrients were significantly higher in the topsoil of the DLB treatment for P_{CAL} and for all soil depths of the DLB treatment for K_{CAL} (Table 2), with no significant differences between year 1 and year 2. Soil mineral N concentrations in spring were significantly higher in the amelioration depth of the DLG treatment, but did not differ significantly among treatments at anthesis (Table 2). Also, spring N_{min} concentrations differed between experimental years in the topsoil and the deeper subsoil, with a decrease from year 1 to year 2 in the topsoil and an increase from year 1 to year 2 in the deeper subsoil.

Table 2 Organic carbon, total N, P_{CAL} and K_{CAL} stocks in soil after equivalent soil mass correction using soil density data (Table S5 and S6, Supplementary Material) based on von Haden et al. (2020) (for non-corrected values see Table S4 in supplementary material) as well as N_{min} concentration in soil of the experimental field for the control (C), and treatments deep loosening (DL), deep loosening with incorporation of biowaste compost (DLB) and deep loosening with incorporation of green waste compost (DLG). Spring N_{min} values were determined in April of the respective year, while all other parameters were measured at flowering. All samples were obtained from the center of the plot above the amelioration furrow. Values are given as mean ± standard error (in parentheses) per treatment and year 1 (Y1, spring barley) and year 2 (Y2, winter wheat) after subsoil amelioration, respectively. Note that due to the experimental design for C, DL and DLB n = 6 and for DLG n = 3 (see methods section). Different letters indicate significant differences ($p \leq 0.05$) among treatments across both experimental years. The p values given in the last column indicate significant difference among experimental years across all treatments and control.

	C			DL			DLB			DLG			*p value*
	Y1	**Y2**		**Y1**	**Y2**[2]		**Y1**	**Y2**		**Y1**	**Y2**		***Y1-Y2***
Organic C [t ha^{-1}]													
0-30 cm	17.0 (0.6)	17.7 (0.7)	A	16.1 [1] (0.4)	16.9 [2] (1.0)	A	21.8 (2.0)	31.9 (5.7)	B	21.7 (1.5)	22.7 (1.2)	AB	n.s.
30-60 cm	6.5 (0.3)	6.5 (0.3)	A	5.6 [1] (0.2)	6.6 [2] (0.4)	A	7.8 (0.5)	10.6 (1.5)	B	7.1 (0.5)	8.4 (0.8)	AB	< 0.05
60-100 cm	9.4 (1.1)	8.8 (1.4)		7.1 [1] (0.4)	6.7 [2] (0.7)		7.5 (0.5)	6.9 (0.7)		6.3 (0.2)	5.9 (0.0)		n.s.
Total N [t ha^{-1}]													
0-30 cm	1.9 (0.1)	2.3 (0.3)		1.8 [1] (0.0)	1.9 [2] (0.1)		2.3 (0.2)	3.0 (0.6)		2.1 (0.1)	2.3 (0.1)		n.s.
30-60 cm	0.9 (0.0)	0.8 (0.0)		0.8 [1] (0.0)	0.9 [2] (0.0)		0.9 (0.1)	1.2 (0.1)		0.9 (0.0)	1.0 (0.1)		< 0.05
60-100 cm	1.3 (0.1)	1.2 (0.1)		1.1 [1] (0.0)	1.0 [2] (0.0)		1.1 (0.0)	1.1 (0.0)		1.0 (0.0)	1.0 (0.0)		n.s.
P_{CAL} [kg ha^{-1}]													
0-30 cm	369 (11)	426 (23)	AB	321 (26)	359 [2] (50)	A	367 (16)	550 (75)	B	302 (38)	338 (31)	AB	n.s.
30-60 cm	44 (7)	75 (10)		48 (7)	103 [2] (41)		79 (12)	107 (20)		46 (10)	58 (13)		n.s.
60-100 cm	31 (4)	62 (13)		30 (3)	23 [2] (4)		38 (7)	74 (31)		30 (2)	43 (4)		n.s.
K_{CAL} [kg ha^{-1}]													
0-30 cm	414 (24)	387 (27)	A	454 (28)	424 [2] (86)	A	634 (47)	916 (170)	B	606 (33)	312 (85)	AB	n.s.

30-60 cm	108 (8)	115 (13)	A	119 (8)	111 [2] (29)	AB	194 (20)	288 (100)	B	158 (22)	87 (24)	AB	n.s.
60-100 cm	163 (15)	185 (17)	AB	151 (12)	85 [2] (28)	A	177 (06)	187 (16)	B	205 (38)	152 (9)	AB	< 0.05
Nmin (spring) [mg kg^{-1}]													
0-30 cm	5.1 [1] (0.4)	2.5 (0.2)		5.3 (0.4)	2.5 (0.2)		5.0 (0.6)	2.6 (0.9)		4.7 (0.4)	2.5 (0.3)		< 0.001
30-60 cm	1.1 [1] (0.0)	1.1 (0.2)	AB	1.8 (0.3)	1.3 (0.1)	A	1.5 (0.1)	1.1 (0.1)	A	1.8 (0.0)	1.6 (0.2)	B	n.s.
60-100 cm	0.8 [1] (0.0)	2.7 (0.4)		1.1 (0.1)	2.8 (0.5)		1.2 (0.3)	3.3 (0.3)		1.0 (0.1)	4.4 (0.6)		<0.001
Nmin (anthesis) [mg kg^{-1}]													
0-30 cm	1.9 (0.2)	2.2 (0.2)		2.2 (0.2)	2.6 (0.3)		2.1 (0.3)	3.4 (0.9)		1.1 (0.4)	2.2 (0.3)		n.s.
30-60 cm	0.5 (0.1)	0.8 (0.2)		0.7 (0.3)	0.7 (0.1)		0.5 (0.1)	0.7 (0.1)		0.2 (0.0)	0.7 (0.2)		n.s.
60-100 cm	0.6 (0.2)	0.4 (0.1)		0.5 (0.2)	1.1 (0.6)		0.6 (0.2)	0.8 (0.4)		0.4 (0.2)	0.4 (0.1)		n.s.

[1] n= 3 due to missing data; [2] n= 4 due to missing data

3.2 Bacteria, archaea and fungi

Gene copy numbers as indicators of the abundance of bacteria, archaea and fungi generally decreased with soil depth (Table S7, Supplementary Material). Especially in the first year after amelioration (CF1-3 2019), microbial biomass (sum of bacterial, archaeal and fungal abundance) was high both in the DL and the DLB treatments, though gene copy numbers were still highly variable across treatments and thus not significantly different from the values of the control in almost all depth intervals.

3.3 Root growth

Root length density at the beginning of the shoot elongation stage in the first year after amelioration was similar among treatments (Figure 1A and B). At anthesis, RLD was higher in the amelioration depth of the DLB treatment compared to the control, both in the first and in the second year after amelioration (Figure 1C-F). However, this effect was significant only for one subtrial in the first year after amelioration (Figure 1D) and the enhanced root growth was confined to the area of the amelioration furrow, while root growth in the surrounding soil (up to 35 cm on either side of the furrow) was not different from root growth in the control plots (graphs for “near” in Figure 1).

There were no significant differences in maximum rooting depth in any year after amelioration (Table S8 Supplementary Material). However, when considering cumulative root length, spring barley in the DLB treatment had developed a larger proportion of its roots in deeper soil layers at the beginning of shoot development (red lines in Figure S2A and B, Supplementary Material). At anthesis, the fraction of deeper roots was reduced to a similar amount as in the other treatments as the main root biomass was then concentrated in the topsoil and upper subsoil until 60 cm depth, both for spring barley and for winter wheat (Figure S2C-F, Supplementary Material).

3.4 Shoot properties at anthesis

At anthesis, LAI and shoot nutrient stocks were significantly higher in the plants of the DLB treatment, while biomass dry matter did not statistically significantly among treatments (Table 3). In contrast, the DL and DLG treatment tended to have lower nutrient uptake as the non-ameliorated control (not significant). Biomass, LAI and shoot nutrient stocks at anthesis were always (except for shoot K stocks) significantly higher in year 2 (winter wheat) than in year 1 (spring barley). When calculating utilization efficiencies for N (NUE), P (PUE) and K (KUE), the DLB treatment had the lowest utilization efficiency in three out of four sampled subtrials, only for spring barley in CF1-2 (2018) the utilization efficiency of the DL treatment was lower than that of DLB (Table S10, Supplementary Materials). However, overall only NUE in the DLB treatment was significantly lower than in the DLG treatment and both NUE and KUE of winter wheat were significantly lower than in spring barley, while all other effects were not significant.

Isotope values for C, N, and Mg in above ground biomass (flag leaf or ear) did not differ significantly among the amelioration treatments (Table 3), although $\delta^{15}N$ values tended to be higher in the DLB and DLG treatments than in the DL treatment and control. There were differences between experimental years with significantly less negative $\delta^{13}C$ and $\delta^{26}Mg$ values for winter wheat than for spring barley and more positive values for $\delta^{15}N$ in winter wheat than spring barley (not significant). For $\delta^{56}Fe$ values, crop specific differences from year 1 to year 2 were not significant. However, here significant treatment effects were observed, with lowest delta values in the DL treatment and highest delta values in the control (Table 3).

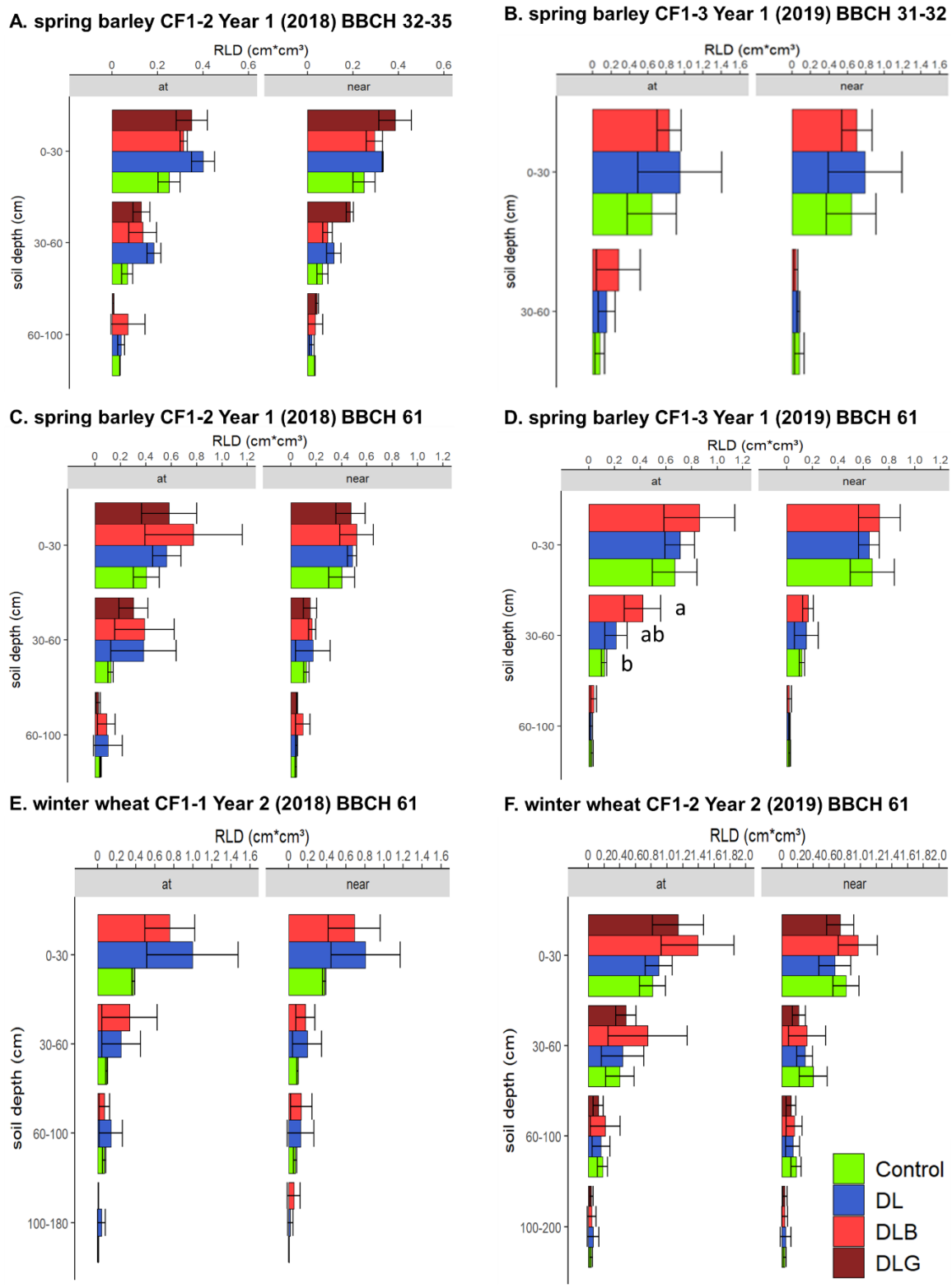

Figure 1 Mean root-length density as recorded with the profile wall method. A. spring barley CF1-2 2018 BBCH 32-35, B. spring barley CF1-3 2019 BBCH 32, C. spring barley CF1-2 2018 BBCH 61, D. spring barley CF1-3 2019 BBCH 61, E. Winter wheat CF1-1 2018 BBCH 61 (data previously published in Jakobs et al. (2019)), F. Winter wheat CF1-2 2019 BCCH 61. Left in each panel: root-length density in soil within the 30 cm amelioration furrow, right in each panel: root-length density in soil up to 35 cm on both sides of the amelioration furrow. Different letters indicate significant differences between treatments at the respective depth increment (ANOVA

followed by Tukey-Test, $p < 0.05$). Data for control is from complete plot and was inserted in columns „at“ and „near“ amelioration to enable comparison to treatment effects. DL: deep loosening, DLB: deep loosening with biowaste compost incorporation, DLG: deep loosening with green waste compost incorporation, RLD: root-length density. Data from plot E has already been shown in Jakobs et al. (2019).

Table 3 Shoot biomass, shoot nutrient stocks and isotope values for plants sampled at flowering in the control (C), and treatments deep loosening (DL), deep loosening with incorporation of biowaste compost (DLB) and deep loosening with incorporation of green waste compost (DLG). All samples were obtained from a 50 x 50 cm square in the center of each plot including area above and next to the amelioration furrow. Values are given as mean ± standard error (in parentheses) per treatment and year 1 (Y1, spring barley) and year 2 (Y2, winter wheat) after subsoil amelioration, respectively. Note that for C, DL and DLB n = 6 and for DLG n = 3 (see methods section). Different letters indicate significant differences ($p \leq 0.05$) among treatments across both experimental years. The p values given in the last column indicate significant difference among experimental years across all treatments and control.

	C			DL			DLB			DLG			*p value*
	Y1	**Y2**		**Y1**	**Y2**		**Y1**	**Y2**		**Y1**	**Y2**		***Y1-Y2***
Biomass													
Dry mass [kg ha^{-1}]	7.9 (0.6)	12.5 (1.1)		7.8 (1.0)	10.5 (1.1)		10.4 (0.5)	11.9 (0.5)		8.7 (0.2)	13.6 (1.7)		< 0.001
LAI [m^2 m^{-2}]	3.5 (0.9)	3.7 (0.5)	A	3.5 (0.8)	3.5 (0.3)	A	4.0 (1.0)	5.3 (0.6)	B	5.3 (0.1)	4.1 (0.5)	A	<0.01
Shoot nutrient stocks													
N [kg ha^{-1}]	90 (13)	143 (13)	AB	88 (9)	127 (12)	A	127 (10)	153 (15)	B	105 (45)	125 (38)	AB	< 0.01
P [kg ha^{-1}]	16 (2)	24 (2)	AB	15 (1)	21 (3)	A	22 (1)	26 (2)	B	19 (1)	27 (2)	AB	< 0.001
K [kg ha^{-1}]	143 (20)	291 (28)	AB	133 (14)	254 (35)	A	197 (12)	345 (72)	B	171 (2)	206 (16)	AB	n.s.
Isotope values													
$\delta^{13}C_{leaf}$ [‰][1]	-29.9 (0.0)	-28.0 (0.5)		-29.6 (0.3)	-27.7 (0.7)		-29.8 (0.2)	-28.0 (0.7)		-29.8 (0.0)	-29.7 (0.2)		< 0.05
$\delta^{15}N_{leaf}$ [‰][1]	0.68 (0.05)	1.41 (0.36)		0.94 (0.19)	1.21 (0.23)		1.09 (0.18)	1.70 (0.25)		1.34 (0.22)	0.55 (0.04)		n.s.
$\delta^{26}Mg_{ear}$ [‰][2,3]	-0.79 (0.02)	-0.46 (0.04)		-0.75 (0.00)	-0.48 (0.03)		-0.78 (0.01)	-0.49 (0.09)		-0.70 (0.02)	NA		< 0.001
$\delta^{56}Fe_{ear}$ [‰][4]	0.54 (0.01)	0.59 (0.01)	A	0.44 (-)	0.26 (0.04)	B	0.50 (0.03)	0.41 (0.03)	AB	0.45 (0.04)	NA	AB	n.s.

[1] n = 3 for all data in Y1; [2] n = 2 for Control in Y1 and n = 3 for all other treatments and years; [3] data previously published in Uhlig et al. (2022) and Uhlig (2022); NA = data not available; [4] n = 1 for DL in Y1, n = 2 for control, DLB and DLG in Y1

3.5 Yield and grain quality

Soil amelioration significantly increased grain yield in the DLB treatment (Figure 2). The DLG treatment only showed higher yields than the control in year 2 (not significant), while in year 1 grain yield was even slightly lower. Straw dry matter and consequently also total dry matter (as the sum of grain and straw dry matter) showed similar trends (Table S11, Supplementary Material). Grain water content and grain fiber content did not differ significantly among treatments or experimental years. Grain protein content was significantly higher in the DLB treatment than in the DL treatment and, additionally, grain protein and grain starch content were significantly larger in spring barley grown in year 1 than in winter wheat grown in year 2 after subsoil amelioration (Table S11, Supplementary Material).

For all four experimental subtrials, the DLB treatment was clearly separated from the control or DL and DLG treatments in the PCA (Figure S3, Supplementary Material). The first two principal components explained more than 60% of the variation in the data, respectively, but were not clearly and repeatedly associated to specific contribution of soil or plant parameters across all four experimental subtrials individually or together. For the two experimental subtrials analyzed in the first year after amelioration (CF1-2 in 2018 and CF1-3 in 2019), yield parameters were most closely related to plant available P content in the amelioration depth, while in the second year after amelioration (CF1-2 in 2019 and CF1-1 in 2018) yield parameters were further closely related to LAI and other soil nutrients.

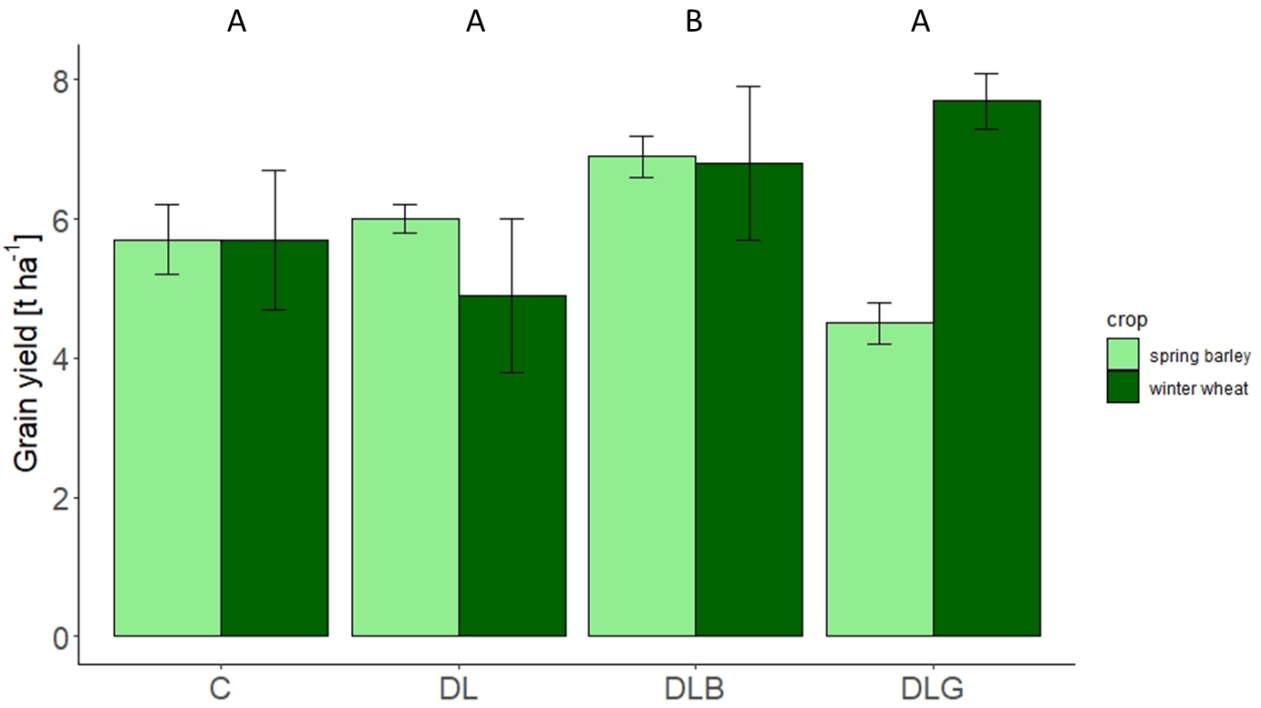

Figure 2 Grain yields determined in manual harvests in the center of the control plot (C) and on the amelioration furrow of the treatments with deep loosening (DL), deep loosening with incorporation of biowaste compost (DLB) or incorporation of green waste compost (DLG). Bars indicate mean values (n=6 for C, DL and DLB, n=3 for DLG; see methods section), error bars denote standard error of the mean. Different letters indicate significant differences among treatments across both experimental years after subsoil amelioration.

4. DISCUSSION

4.1 Effect of subsoil amelioration by mechanical loosening

Subsoil amelioration in Germany primarily aims at removing pedogenically dense or anthropogenically compacted root restricting layers (Schneider and Don, 2019), i.e., decreasing soil bulk density, which will increase the rootability of the soil and thereby enhance the accessibility of subsoil resources for crop production. Yet, in reported literature, subsoil amelioration does not consistently result in the aforementioned beneficial effects, sometimes even the opposite of the intended effects is observed (Sale et al., 2019; Schneider et al., 2017).

For the soil at our experimental site, clay accumulation in the subsoil has been observed (Bt horizon), but when taking into account the bulk density and texture of the subsoil in the control plots, the subsoil compactness was still below the level that is considered as limiting for crop root growth as indicated for German soils by Schneider and Don (2019). In our experiments, covering two years after subsoil amelioration, the highest values of subsoil bulk density in the amelioration depth were observed when the soil was mechanically loosened without addition of organic amendments (DL treatment, differences to other treatments or control not significant; Table 2). Higher bulk densities after amelioration by mechanical loosening have been observed in previous studies and have been attributed to a re-compaction of the soil after the loosening, potentially related to a collapse of the original soil structure (Larney and Fortune, 1986; Schneider et al. 2017). However, this could not be confirmed here given the lack of statistical significance in our experiment. A significantly lower bulk density was only observed in the deeper subsoil of the DL treatment. This can, however, not be attributed directly to the soil amelioration procedure as it occurred at a depth below the reach of the amelioration tine and likely reflected local heterogeneity or local loosening of the soil below the amelioration depth by deep roots of the crops. Mechanical subsoil amelioration thus did not produce the intended loosening effect, but nevertheless bulk density was not a limiting factor for root growth in the subsoil at our experimental site.

With the mechanical loosening of the subsoil in the way that it was implemented in the DL treatments of our experiments (see methods description), topsoil and subsoil were not mixed and no organic material was introduced into the subsoil. Therefore, also subsoil soil organic C and total N stocks were not significantly

affected by the loosening procedure. Nevertheless, marginally higher nutrient availability as indicated by P_{CAL} and K_{CAL} stocks and (spring) N_{min} concentration was observed in the DL treatment, although this effect was again not significant. For the soil at our site, high stocks of nutrients in the subsoil had been previously reported in other studies (compare, e.g., Barej et al., 2014; Bauke et al., 2017; Seidel et al., 2019). Hence, we suggest that the temporary loosening and aeration of the subsoil may have induced a transient mineralization flush of these nutrients. Yet, none of the microbial parameters (bacteria, archaea and fungi) analyzed in this study indicated a significant increase in microbial abundance in the subsoil of the DL treatment, which would have supported nutrient mineralization processes. Therefore, considering the lack of statistical significance, the overall relevance of mechanical subsoil loosening for nutrient mineralization and availability for crop production remains unclear.

A removal of root restricting layers in the subsoil facilitates deeper rooting and enables exploration of a larger soil volume by crop roots (Han et al., 2021; Schneider and Don, 2019; Thorup-Kristensen et al., 2020). Here, even with no root-restricting layers present and no significant effect on soil bulk density, root length density was enhanced in the DL treatment in the first year after amelioration, although this effect was not significant as it was not consistently observed across experimental subtrials (compare Figure 1C and D). The response of the roots to the subsoil loosening may thus have been more sensitive than our measurements of soil bulk density.

The enhanced root growth at the amelioration depth did not result in overall increased resource uptake (Table 3). Nitrogen, P and K stocks in above ground biomass and nutrient utilization efficiencies were not significantly different among the DL treatment and the control (Table 3 and Table S10, Supplementary Material). Interestingly, deep loosening further increased root length density in the second year after amelioration in the topsoil (not significant, Figure 1E), hence above the intended amelioration depth of 30 – 60 cm. Consequently, more root surface area was available for nutrient uptake in the topsoil horizon compared to the control. This shallower uptake depth was also observed in a companion study based on the same samples, in which the isotope ratio $^{87}Sr/^{86}Sr$ was used as a proxy for the uptake depth of mineral nutrients (Uhlig et al. 2023). In our study, isotopic indicators in plant biomass, such as $\delta^{13}C$ values, which, among other factors, indirectly reflect water use efficiency (Farquhar et al., 1989), $\delta^{15}N$ values as an indicator of fertilizer NUE

(Chalk, 2018; Kriszan et al., 2009) or δ^{26}Mg as an indicator for Mg uptake (Uhlig et al., 2022; Wang et al., 2020) did not show any significant effect of the DL treatment. Conversely, the δ^{56}Fe values of wheat and barley ears were shifted to significantly less positive values in the DL treatment compared to the control. While soils typically have high total Fe content, the plant available Fe pool is usually very small due to the limited solubility of most Fe compounds found in soils. Hence, changes affecting the plant available Fe pool might also affect the δ^{56}Fe values of the plant organs (Wu et al., 2019, 2021). However, the standard deviation of repeated measurements was high (Table S9, Supplementary Material), with double standard deviation (2SD) ranging from 0.12 to 0.19‰ and thus complicating the assessment of different Fe uptake strategies by mere monitoring of δ^{56}Fe values in plants. Here, likely more sophisticated analyses of different pools in soil and subsequent modelling is needed as shown recently for Mg (Uhlig et al., 2022).

With regard to biomass production, dry biomass and LAI at anthesis (Table 3) as well as final grain yield (Figure 2) and grain quality (Table S11, Supplementary Material) no significant influence by the mechanical loosening of the subsoil was observed. In summary, we thus have to refute our first hypothesis that mechanical loosening of the subsoil has short-term positive effects on soil physical properties such as bulk density. Also, no significant effect on soil chemical properties was observed and incremental effects on root growth did not result in overall enhanced crop performance.

4.2 Effect of subsoil amelioration by mechanical loosening with incorporation of organic matter

As opposed to only mechanical loosening, the incorporation of organic matter into the subsoil was intended to both maintain the loosened soil structure and provide a reservoir of water and nutrients accessible to crop roots. In line with this expectation, subsoil bulk density was significantly reduced in the DLB and DLG treatments compared to the control, i.e., in those treatments where organic matter was incorporated into the subsoil upon loosening (Table 1). Thus, for the given experimental site with silt-loam soil and clay accumulation in the subsoil, a persisting effect of subsoil amelioration was only achieved when organic amendments were added to the subsoil to stabilize the loosening effect (Getahun et al., 2018; Jayawardane et al., 1995). Previous studies have suggested that such stabilization may occur due to the role of organic matter in aggregate formation

(Amelung et al., 2023; Bronick and Lal, 2005; De Gryze et al., 2006), but this cannot be confirmed for the presented study as aggregate size fractions were not analyzed.

The amendments further added substantial amounts of organic material to the subsoil, as evident from significantly elevated C stocks but not N stocks in the DLB treatments (Table 2). Considering the application amount of 50 kg m^{-1} along the furrow with the element concentrations listed in Table S2, this application should result in a C addition of 8.5 kg m^{-1} in the DLB treatment and 8.9 kg m^{-1} in the DLG treatment, which is equal to an application rate of 28.5 and 29.7 kg m^{-2} within the area of the furrow, respectively. The corresponding N addition amounted to 635 g m^{-1} in DLB and 334 g m^{-1} in DLG, equal to 2.1 and 1.1 kg m^{-2} within the furrow area, respectively. Total element addition was therefore rather low, which resulted in significant changes in soil element stocks for C but not for N.

The addition of organic matter into the loosened subsoil also resulted in significantly increased pH and electrical conductivity in the amelioration depth of the DLB treatment (Table 1), which may have contributed to the observed changes in P and K availability. The stocks of plant-available K_{CAL} were significantly increased, while the corresponding values for P (P_{CAL}) were not significantly different from the control. As opposed to K, P availability is sensitive to changes in pH and the lack of a significant increase in P availability after organic matter addition may be due to the increased pH, which should result in lower P availability in soil solution (e.g., Penn and Camberato, 2019).

The concentration of N_{min} in the amelioration depth was significantly higher in the DLG treatment in spring, but not at anthesis, and not at all in the DLB treatment. This observation was not expected, as the green waste compost was characterized by a wider C:N ratio (Table S2), thus potentially promoting microbial N immobilization (Bengtsson et al., 2003; Janssen, 1996). Additionally, N_{min} appeared to be redistributed within the soil profile over the course of the two years. Spring N_{min} concentrations were initially highest in the topsoil, which likely resulted from the yellow mustard cover crop that had been grown during the winter months of year 1 to allow for the soil to rest between subsoil amelioration and sowing of spring barley (see experimental time line in Figure S1, Supplementary Material). By the spring of the second year, the highest N_{min} concentrations were then observed in the deep subsoil below the amelioration depth. This likely again derived from N mineralized during the winter months. Considering that winter wheat was already sown in the previous

654 fall and thus further advanced in growth compared to the spring barley at the time of sampling in spring, N
655 mineralized in the upper soil layers may have already been taken up by the winter wheat at the time of
656 sampling. Increased Nmin concentrations were thus only observed in the deeper subsoil, which was not reached
657 by the roots yet. However, in both experimental years, N_{min} concentrations in the deeper subsoil at anthesis
658 were low, suggesting that either N was leached below the deepest sampling depth or that mineralized N was
659 effectively used by the crops. It should also be noted that this pattern was observed in all treatments and the
660 control, thus our data do not indicate increased N mineralization and leaching risk after the incorporation of
661 organic matter, compared to standard soil management or only mechanical loosening. Overall, the results thus
662 only partially support our second hypothesis that subsoil loosening with simultaneous incorporation of organic
663 matter enhances nutrient availability, especially when biowaste compost was used.

664 Immobilization of nutrients in microbial biomass can contribute to the mitigation of nutrients added with the
665 organic amendments. 16S and 18S rRNA gene copy numbers as indicators of microbial biomass were slightly
666 (not significant) enhanced in the DLB treatments, but not in the DLG treatment (Table S7, Supplementary
667 Material), suggesting that microbial communities mainly thrived on the initial input of nutrients and easily
668 available C (Lv et al., 2022; Mooshammer et al., 2014). It would have been expected that the biowaste compost
669 with narrow C:N ratio would provide a substrate that can be easily mineralized and immediately stimulate
670 microbial growth, while the green waste compost with a wider C:N ratio would be mineralized more slowly,
671 showing stronger effects in the second year than the first. However, this assumption was not supported by the
672 microbial biomass data in our experiment. Given the lack of statistical power, we are therefore not able to
673 conclusively determine whether and to what extent microbial nutrient mineralization or immobilization
674 contributed to the observed patterns in available nutrients.

675 Improved access to subsoil resources, for example via pores created by deep rooting pre-crops, has been shown
676 to enhance nutrient (Han et al., 2021; Seidel et al., 2019) and water (Gaiser et al., 2012) uptake from the
677 subsoil. Similarly, Sale et al. (2019) observed higher subsoil water extraction after subsoil loosening and
678 incorporation of poultry manure, which was attributed to deeper root growth. In our experiment, only the DLB
679 treatment induced enhanced RLD and higher proportions of roots in the subsoil at the amelioration depth,
680 similar to previous experiments (Gill et al., 2009). However, this effect was only noticeable in the ameliorated

area, but did not stimulate root proliferation into the surrounding areas or deeper subsoil. We did not specifically evaluate soil water contents in this study. Nevertheless, for the subtrial CF1-1 in 2018 (which was also included here) a previous study by Jakobs et al. (2019) observed lowest soil water contents at the immediately underneath the amelioration furrow compared to the control and the DL treatment, pointing to enhanced water extraction. We therefore suggest that the combined effect of reduced soil density and enhanced nutrient availability as in the ameliorated furrow of the DLB treatment stimulated root growth. By comparison, despite similarly reduced bulk density, the slower mineralization of organic matter in the DLG treatment resulted in less pronounced effects on root growth in the first two years after organic matter incorporation (Figure 1 and Table S8, Supplementary Material).

The observed differences in root growth further define resource acquisition from the soil. Accordingly, biomass and LAI as well as nutrient contents of plants at anthesis were significantly enhanced in the DLB treatment, but not in the DLG treatment in the first two years after subsoil amelioration (Table 3). Thus, it can be assumed that the enhanced root growth into the ameliorated subsoil in the DLB treatment was the main cause for the higher crop nutrient (and potentially water) acquisition from the subsoil. This highlights the potential of subsoil resources in mitigating drought impacts during the critical phases of anthesis and yield formation. The increase in nutrient uptake in the DLB treatment, however, induced a lower utilization efficiency, especially for N and K, compared to the control. Similarly, isotopic indicators of the flag leaf at anthesis showed a trend (not significant) for higher $\delta^{15}N$ values in DLB compared to the control (Table 3), pointing to high levels of isotopic discrimination prior to N uptake, and thus lower fertilizer NUE (Chalk, 2018; Kriszan et al., 2009) compared to the other treatments. By comparison, $\delta^{13}C$ values did not differ among treatments. Possibly, improved water supply and thus higher water uptake (i.e., lower water use efficiency) were compensated by overall greater water loss due to higher transpiration rates with elevated biomass production; however, such processes could not be disentangled with the analyses performed here.

Similar to $\delta^{15}N$, stable isotope ratios of Mg and possibly also Fe can be used as an indicator for nutrient use efficiency and uptake strategies (for recent evidence for Mg, see e.g., Uhlig et al., 2022 and Wang et al., 2020). However, our data did not indicate that deep loosening with or without the addition of biowaste compost or green waste compost had a significant net effect on the $\delta^{26}Mg$ values of wheat and barley ears, likely because

additions of lime affected δ^{26}Mg isotope ratios (Uhlig et al., 2022; Wang et al., 2020). In contrast, recent monitoring of $^{87}Sr/^{86}Sr$ isotope ratios provided clear evidence of the additional uptake from geogenic nutrient sources in the DLB treatment (Uhlig et al., 2023), thus supporting the idea that this treatment improves resource use.

In summary, enhanced plant growth and nutrient uptake after subsoil amelioration supported our third hypothesis, although we were not conclusively able to attribute this to a specific change in soil physical or chemical conditions. Further, the magnitude of these effects depended on the type of organic matter amendments, with biowaste compost inducing more immediate positive effects for crop growth than did green waste compost. Nevertheless, it has to be considered that the overall higher supply of resources was not used efficiently by the plants, which is a common observation for crops grown on soil with higher nutrient supply than required (see, e.g. Rose et al., 2016; Weih et al., 2018) and suggests that compost amounts might need to be adjusted to avoid oversupply.

As a consequence of the overall positive effects of subsoil loosening and biowaste compost addition on nutrient concentration and availability in the soil, root growth and crop development, also overall grain yields were significantly higher in the DLB treatment than in the control across all years (Figure 2). Similar observations were already reported in earlier studies showing higher yields after incorporation of organic matter into the subsoil (Getahun et al., 2018; Jakobs et al., 2019, Ma et al., 2009; Schmittmann et al., 2021), but yield increases in the DLB treatment were lower than reported in some other studies (e.g. Sale et al., 2019; Uddin et al., 2022). We suggest that in addition to climatic factors (Ma et al., 2009), the magnitude of the yield increase depends on the strength and type of the initially limiting factor as well as the type of organic material used for subsoil amelioration. In our experiment, soil fertility was already high before amelioration and bulk density was below critical levels for root growth. Additionally, both the biowaste and green waste compost have lower nutrient concentration than materials used in other studies, such as poultry manure (McPhee et al., 2023; Sale et al., 2019; Uddin et al., 2022). Noteworthy, the DLB treatment generally also significantly enhanced grain protein contents. Nevertheless, grain quality parameters were in a good to very good range of protein contents required for downstream production processes, thus confirming our fourth hypothesis that organic matter amendments to the subsoil can increase both yield quantity and grain quality. By comparison, grain yield in the DLG

735 treatments were enhanced only in the second year (winter wheat), suggesting that organic subsoil amendments
736 with wider C:N ratio may have longer response times in providing beneficial effects for the crops than the
737 biowaste compost. Longer observation periods than the two years after subsoil amelioration studied here are
738 now needed to evaluate these effects in the long-term.

739

740 ## 5. CONCLUSION

741 In our experiments, despite clay accumulation in the subsoil, soil bulk density was not an initially limiting
742 factor for plant and root growth and mechanical loosening alone did not provide beneficial effects for root
743 growth and crop development. By comparison, addition of biowaste compost into the loosened subsoil resulted
744 in immediate positive effects on crop performance, demonstrating the short-term potential of subsoil loosening
745 with admixture of organic amendments in cropping years when dry periods occur during critical phases of
746 yield formation. The addition of green waste compost had less pronounced effects in the first two years after
747 amelioration, but might still become beneficial over longer time scales. Noteworthy, we were not able to
748 attribute the higher yields after subsoil amelioration with biowaste addition to any specific change in soil or
749 microbial parameters. The enhanced crop development thus likely resulted from the combined effect of
750 changes in physical, chemical and biological soil properties, with a more detailed analysis of the underlying
751 interactions still warranting further attention.

752

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757

758 ## DECLARATIONS

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