



# Effects of conventional, organic and conservation agriculture on soil physical properties, root growth and microbial habitats in a long-term field experiment

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## ABSTRACT

Soil structure is important for plant growth and ecosystem functioning, and provides habitat for a wide range of soil biota. So far, very few studies directly compared the effects of three main farming practices (conventional, organic and conservation agriculture) on soil structure and soil physical properties. Here, we collected undisturbed soil cores from the Farming System and Tillage long-term field experiment (FAST) near Zurich (Switzerland). This trial compares the effects of conventional tillage, conventional no-tillage, organic tillage and non-inversion reduced tillage under organic farming since 2009. We assessed 28 soil chemical and physical properties and related them to root and microbial biomass as well as to the diversity of bacteria and fungi. Tillage decreased bulk density (−14 %) and penetration resistance (−40 %) compared to no/reduce-tillage, potentially promoting a facilitative environment for plant root growth. Water holding capacity varied among systems, being the lowest in conventional tillage and highest (+10 %) in organic reduced tillage. We observed that microbial biomass and rhizosphere microbial diversity was positively associated with water holding capacity and the occurrence of mesopores. The presence of mesopores could provide additional niche space for microbes possibly explaining its positive effect on microbial diversity. Soil microbial biomass and rhizosphere microbial diversity were higher in plots subjected to soil conservation practices, indicating that tillage has a detrimental effect on soil microbes. Our work demonstrates that organic, conventional and conservation agriculture create contrasting soil physical environments. This work highlights the trade-off between creating a facilitative environment for root growth by tillage and maintaining complex and diverse soil microhabitats for microbes under conservation agriculture.

## 1. Introduction

Agricultural intensification has led to a substantial increase in food production over the past century. However, intensive agriculture can have negative impacts on the environment, in terms of biodiversity loss, increased greenhouse gas (GHG) emissions, consumptive use of freshwater, water pollution due to pesticides and fertilizers, soil erosion, and soil degradation (Pretty et al., 2018). Once degraded, soil recovery is an extremely slow process (Lal, 2015). According to the Food and Agriculture Organization of the United Nations (FAO, 2015), 33 percent of

the world's soils are moderately to highly degraded, which jeopardizes food production and food security. This threat could be counteracted by the implementation of sustainable soil management practices (FAO, 2015). Designing arable cropping systems that align soil quality and productivity demands is one effective way of preserving the soil as a resource and as a provider of many functions and services that we all depend on (Pretty et al., 2018; Tamburini et al., 2020).

A range of agricultural practices are thought to have a reduced impact on the environment and these include organic farming (Krauss et al., 2020; Winqvist et al., 2012) and conservation agriculture (Hobbs

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et al., 2008; Wittwer et al., 2021). Organic agriculture focuses on adaptation to local resources and conditions, limiting external inputs such as chemical fertilizers and pesticides (El-Hage Scialabba, 2013) and building on natural soil fertility. Conservation agriculture relies on the three principles of 1) crop diversification, 2) permanent soil cover, and 3) minimal soil disturbance to achieve soil protection and a better use of natural resources (FAO, 2022).

Soil conservation practices, under both organic and conservation agriculture, have the potential to positively impact important soil structure-mediated ecosystem functions (Wittwer et al., 2021) such as carbon sequestration, climate regulation, water purification, nutrient cycling and erosion risk reduction (Fabrizzi et al., 2005; Giambalvo et al., 2018; Loaiza Puerta et al., 2018; Norris and Congreves, 2018; Schlüter et al., 2018; Seitz et al., 2018). However, these positive effects are variable and depend on the context and local conditions (Autret et al., 2020). For example, N losses can be large in organic farming, especially if related to the amount of produced crop (Kirchmann and Bergström, 2001). Despite a different depth distribution of SOC and an enrichment in the topsoil layer, total carbon stocks are often not much higher in no-tilled than tilled systems (Baker et al., 2007; Krauss et al., 2022; Ogle et al., 2019). No-tilled topsoils are often denser than tilled ones, which may reduce soil gas transport capabilities and impair root growth for certain crops (Martínez et al., 2016).

Moreover, conservation agriculture and organic farming seem to present a trade-off between being environmentally friendly vs. highly productive (Reganold and Wachter, 2016). Regarding productivity, responses to tillage intensities are ambiguous, and could vary within cropping systems contexts such as climate (Preusser et al., 2017; Rovira and Vallejo, 1997), time since system conversion (Blanco-Canqui and Wortmann, 2020), and crop rotation (Schlüter et al., 2018). On average, no-tillage systems have been shown to reduce yields by about 6 percent when compared to conventional tillage (Pittelkow et al., 2015b), although this effect may vary according to the type of crop, soil texture, climate (Hartmann and Six, 2023; Pittelkow et al., 2015a), and nitrogen fertilization level (MacLaren et al., 2022). Organic farming yields are on average 19 percent lower than those under conventional practices (Ponisio et al., 2015), and organic farming has a reduced yield stability (Knapp and van der Heijden, 2018).

Tillage has been applied for millennia to loosen soil and improve crop growth conditions, control weeds and pathogens, incorporate crop residues, and promote nutrient mineralization and availability (Hobbs et al., 2008). This is why organic farmers often apply inversion tillage as a tool to incorporate organic material into the soil and control weeds and pathogens, since no chemical control is allowed (Vakali et al., 2011). Although tillage of soil brings certain benefits for crop productivity, these advantages come at an environmental cost. Inversion tillage is associated with risks, including: I. the release of CO<sub>2</sub> to the atmosphere due to the mechanical action of breaking up soil and increased microbial respiration (Mangalassery et al., 2014; Reicosky, 1997), II. indirect GHG emissions associated with diesel consumption during tillage, III. disruption of soil pores that could have negative effects on water infiltration (Abid and Lal, 2009), IV. destabilization of soil structure increasing risk of erosion by water and wind (Seitz et al., 2018), and finally (V), negative effects on soil organisms (Crittenden et al., 2014; Degrunne et al., 2017; Säle et al., 2015).

Soil organisms play a pivotal role in governing numerous biochemical and physical processes crucial for soil functionality, including nutrient cycling, carbon sequestration, and water infiltration and retention (Barrios, 2007; Edlinger et al., 2022). The microbial community within roots vicinities, i.e. the rhizosphere, significantly contributes to essential biogeochemical processes. The rhizosphere is a dynamic hotspot comprising microorganisms filtered from the bulk soil (Park et al., 2023; Tkacz et al., 2015), and potentially from the plant endosphere (Berg and Raaijmakers, 2018; Ren et al., 2020). These recruited microorganism play a key role in enhancing plant stress tolerance and nutrient acquisition (Ling et al., 2022).

The rhizosphere microhabitat displays increased microbial activity compared to the bulk soil (Moreau et al., 2019). However; on a broader scale, a structured bulk soil enables the coexistence of an extensive diversity of organisms (Hartmann and Six, 2023; Nielsen et al., 2015), resulting in a large genetic pool that can be tapped into by plant rhizospheres under varying stress conditions (Bakker et al., 2015; Park et al., 2023). Pore size distribution has been shown to affect microbial community composition, associations, and functioning (Sleutel et al., 2012; Xia et al., 2022). Besides contributing to the maintenance of biodiversity, soil structure strongly influences many other soil processes such as mineralization and stabilization of organic carbon, or transport and cycling of nutrients (Kravchenko et al., 2020; Nunan et al., 2017; Schlüter et al., 2020). The soil pore network architecture modulates mass flow (i.e., water, air and organisms), directly influences ecological traits such as root and hyphae development, and governs the physical habitat and conditions (moisture, aeration, temperature) of soil macro-, meso- and microorganisms (Carson et al., 2010). For example, large macro-porosity and high pore connectivity improve soil aeration and decrease penetration resistance, which facilitate plant root growth (Bengough et al., 2011, Kuzyakov and Blagodatskaya, 2015, Colombi et al., 2017, Landl et al., 2017). However, increased pore network connection also exposes less competitive and prey species to more dominant ones, which may decrease soil biodiversity through competitive exclusion (Carson et al., 2010, Negassa et al., 2015, Bickel and Or, 2020). The characteristics of the pore network are susceptible to changes due to soil management activities such as tillage and amendments. Thus, quantifying the modifications of soil structure induced by cropping practices is imperative to evaluate the impact of agricultural systems on the soil environment (Pagliai et al., 2004).

A range of studies has compared impacts of conservation and conventional tillage on soil properties and crop yield (Ashapure et al., 2019; Cooper et al., 2016; Gregory et al., 2007; Krauss et al., 2020; Sommer et al., 2007; Stagnari et al., 2010; Tebrügge and Düring, 1999), and several studies have compared organic with conventional agriculture (Abdollahi et al., 2014; Gerhardt, 1997; Maeder et al., 2002; Puleman et al., 2003; Reganold and Wachter, 2016; van Leeuwen et al., 2015; Zani et al., 2020) suggesting positive effects of mechanical tillage for crop yields, while organic inputs and conservation tillage have a greater potential to contribute towards increased soil health and structure (Alori et al., 2020). However, direct comparison of conventional and organic agriculture under different tillage regimes remain scarce in the literature (Crittenden and de Goede, 2016; Loaiza Puerta et al., 2018; Walder et al., 2023).

It has been shown that the effect of the interacting stressors in a system can be higher than the impact of isolated and single stressors (Rillig et al., 2023). Thus, in this study, we investigated the effects and interactions of tillage and different cropping system on soil physical properties, investigating a set of 28 soil variables. Our study was based on data obtained in a long-term field experiment in Switzerland established in 2009, comparing four important arable cropping systems: conventional farming with inversion tillage, conventional farming under no-tillage, organic farming with inversion tillage, and organic farming with reduced tillage (Wittwer et al., 2021). The objectives of this study were 1) to characterize and quantify the impact of different tillage practices and cropping systems on soil structure and soil quality after eight years of contrasting management, and 2) to identify soil physical properties which facilitate root growth or influence soil microbial biomass and the diversity of rhizosphere microbiota in different cropping systems.

We hypothesized that conventional tillage systems exhibit higher root growth as it is easier for roots to penetrate in disturbed and loosened soil, leading to positive associations between root growth and physical properties conducive to intensive tillage. We further expected that conservation tillage, characterized by minimal soil disturbance and maintenance of soil cover, will promote soil structure stability and create favourable soil microbial habitats, leading to higher microbial

diversity. Additionally, we anticipated that organic farming practices, with a focus on soil conservation, organic inputs and the absence of synthetic pesticide input, will enhance soil water retention and be positively associated with microbial diversity.

## 2. Materials and methods

### 2.1. Field site and soil management

This study was carried out at the FArming Systems and Tillage experiment (FAST), located at Agroscope (Zürich/Switzerland, latitude 47°26'N, longitude 8°31'E). The field experiment was established in 2009 to investigate the agronomical and ecological performance of contrasting arable cropping systems representing conventional, organic and conservation agriculture (Wittwer et al., 2021). The main factor “cropping system” (main plot level) includes four treatments: conventional farming with inversion tillage (C-IT) and no-till (C-NT), and organic farming with inversion tillage (O-IT) and reduced tillage (O-RT). FAST is arranged as split-plot design with four randomized spatial blocks and two temporal replicates (staggered start design, FAST I and FAST II) (Fig. S1). The four cropping systems have the same 6-year crop rotation consisting of winter wheat, grain maize, a grain legume crop, winter wheat, and a 2-year grass-clover ley. In the conventional systems, crops are fertilized with mineral fertilizer, and pesticides are used for weed control and plant protection, whereas slurry is applied in the organic systems, while synthetic fertilizers and pesticides are prohibited. For more details on the field trial, see Wittwer et al. (2021). The inversion tillage systems (O-IT and C-IT) were tilled to a depth of 0.2 m, while organic reduced tillage was performed to a maximum depth of 0.1 m with a disc harrow (2009), rotary harrow (2010–2015), and thereafter with chisel and rototillers at a target depth of 0.05 m. In the conventional no-till system (C-NT), crops were directly sown in the soil in furrows created with a disc opener. Crop residues were kept in the soil as mulch. Chemical weed control was performed in both conventional systems. Mechanical weed control operations (<0.1 m depth) were performed in the organic systems with a star cultivator in row crops and a tin harrow in drilled crops.

### 2.2. Soil sampling

Undisturbed soil cores in aluminum cylinders (0.05 m diameter ( $\emptyset$ ), 0.05 m height; approx.  $10^{-4}$  m<sup>3</sup> volume) were sampled at 0.075–0.125 m soil depth (hereafter 0.1 m) and 0.375–0.425 m soil depth (hereafter 0.4 m) on 04.07.2017, approximately two months after tillage (16.05.2017) and sowing of a maize crop (18.05.2017). We sampled four cores per plot at two depths in four blocks, totaling 16 cores per cropping system and soil depth (128 soil cores in total). We stored the samples at 4 °C until further analysis. All the soil cores were used for measurements of soil organic carbon (SOC), bulk density ( $d_b$ ), gas transport properties (air permeability and gas diffusivity), and water retention at the matric potentials of ( $\Psi$ ) –0.003, –0.01 and –0.03 MPa.

Only two cores per plot (instead of 4) were used for measurements of penetration resistance and water content at –1.5 MPa due to the long-time requirement for those assessments. Two weeks after the sampling of the undisturbed soil cores (19.07.2017), composite soil samples from 0 to 0.1 m depth were collected in each plot, for microbial biomass and pH measurements. On the same date, we sampled four individual maize root stocks per plot for rhizosphere fungal and bacterial diversity assessments. The root stocks from which the rhizosphere was isolated to sequence the microbial community, was frozen and processed as described below in the session “Rhizosphere fungal and bacterial diversity”. The samples collected for microbial biomass assessment were kept in a cold room at 4 °C until analyzed.

### 2.3. Soil characteristics: pH, soil texture and soil organic carbon

Soil pH was measured in water extract, soil texture was determined in water suspension aliquots by the sedimentation analysis and SOC was measured by the wet oxidation method using potassium dichromate according to the Swiss reference method for soil analyses (FAL, 1996).

### 2.4. Soil porosity and plant available water

All undisturbed soil cores were saturated from below and equilibrated to matric potentials of –0.003, –0.01 and –0.03 MPa on ceramic suction plates for the determination of soil water retention. Water content at –1.5 MPa was determined on smaller undisturbed samples (0.01 m height by 0.05 m  $\emptyset$ ) in PVC rings subsampled from the cores used for the penetration resistance assessments (see section 2.2). After all measurements, we oven-dried all cores at 105 °C for at least 24 h and weighed them for bulk density determination. Particle density was measured by the water displacement method and used for the calculation of total porosity. Air-filled porosity was calculated as the difference between total porosity and volumetric water content at the respective matric potential. Pore size distribution was obtained by estimating the equivalent pore diameter  $d$  ( $\mu\text{m}$ ) as  $d = -0.3/\Psi$  (Schjønning et al., 2002). In this study, we consider macropores as pores with  $\emptyset > 30 \mu\text{m}$  (corresponding to matric potentials  $> -0.01$  MPa), mesopores as pores with  $\emptyset$  between 10 and 30  $\mu\text{m}$  (corresponding to matric potentials between –0.03 MPa and –0.01 MPa) and micropores as pores  $\emptyset < 10 \mu\text{m}$ . We further divide macropores into large macropores ( $\emptyset > 100 \mu\text{m}$ ) and small macropores ( $\emptyset$  between 30 and 100  $\mu\text{m}$ ), and micropores into large micropores ( $\emptyset$  between 0.2–10  $\mu\text{m}$ ) and small micropores ( $\emptyset < 0.2 \mu\text{m}$ ). The choice for the 30  $\mu\text{m}$  threshold for macropores is according to Cresswell and Kirkegaard (1995) and reinforced by the study of Yunusa and Newton (2003), who showed that lateral roots of cereals form biopores  $>30 \mu\text{m}$ .

We calculated the amount of maximum plant available water (PAW) as the difference between the volumetric water content at field capacity (here taken as –0.01 MPa) and permanent wilting point (–1.5 MPa) as suggested by Reynolds et al. (2009). Maximum water holding capacity (WHC) was defined as the water content at field capacity (Hardy, 1923).

### 2.5. Soil penetration resistance

We measured penetration resistance by a bench electronic penetrometer, according to a methodology described by Ruiz et al. (2016). A cone was inserted into the soil cores at a speed of  $10 \times 10^{-3}$  m min<sup>-1</sup>. At the matric potential of –0.03 MPa we performed four cone penetrometer insertions, 7 mm into the soil. The cone was connected to a force transducer (LC703, OMEGA Engineering Inc., Stamford, CT, USA) and had a recessed shaft, a semi-apex angle of 15° and a cone base area of  $3 \times 10^{-6}$  m<sup>2</sup>. We calculated the average penetration force for each core, and divided the average force by the cone base area to convert it to soil penetration resistance.

### 2.6. Soil gas transport properties and pore network characteristics

Air permeability ( $K_a$  [ $\mu\text{m}^2$ ]) and the relative gas diffusivity coefficient ( $D_p / D_0$  [–]) were measured for all cores at matric potentials of –0.003, –0.01 and –0.03 MPa according to Martínez et al. (2016). From the gas transport measurements, we calculated the structural parameter (SP; eq. (2) (Kawamoto et al., 2006) and the pore organization (PO; eq. (3) (Groenevelt et al., 1984) indices at –0.01 MPa (field capacity) as follows:

$$SP = \frac{k_a}{D_p/D_0} \quad (2)$$

and

$$PO = \frac{K_a}{\varepsilon_a} \quad (3)$$

These indices provide information on the connectivity, continuity and tortuosity of the pore network but do not allow to distinguish between pore connectivity or tortuosity.

## 2.7. Microbial biomass

We estimated microbial biomass carbon by the chloroform-fumigation-extraction method according to Vance et al. (1987). The extraction was performed in duplicates on 20 g (dry matter) subsamples that were extracted with 80 ml of a 0.5 M K<sub>2</sub>SO<sub>4</sub> solution. Organic C (TOC) was determined by infrared spectrometry after combustion at 850 °C (DIMA-TOC 100, Dimatec, Essen, Germany). Soil microbial biomass was then calculated according to eq. (4).

$$C_{mic} = \frac{E_C}{K_{EC}} \quad (4)$$

Where E<sub>C</sub> = (TOC in fumigated samples – TOC in control samples) and K<sub>EC</sub> = 0.45 (Jørgensen, 1996).

## 2.8. Rhizosphere fungal and bacterial diversity

In total, 64 rhizosphere samples were collected from four maize root stocks per plot, four cropping systems (C-IT, C-NT, O-IT, O-RT), and four blocks, however 6 samples did not yield enough rhizosphere material for microbial analysis (Table S1). Rhizosphere samples were isolated from 10 cm fragments of roots collected from the major rooting zone (0.05–0.15 m soil depth) after shaking the root stock to remove bulk soil. The 10 cm root fragments were further processed into fragments of about 3 cm using sterilized containers and scissors. These root fragments were placed in 50 ml falcon tubes filled with 25 ml of sterile milli-Q water. The tubes were vigorously shaken for 10 times, the water transferred to two other clean falcon tube and this process was repeated four times. Each rhizosphere sample was prepared by combining all four wash fractions (4x25 ml) using centrifugation (5 min at 3220 × g, discarding the supernatant), and the resulting pellets were collected in 1.5 ml microcentrifuge tubes and stored at –20 °C until further use.

Nucleic acids were extracted from 250 mg of rhizosphere using the NucleoSpin Soil DNA extraction kit (Machery-Nagel GmbH & Co. KG, Düren, Germany). In order to maximize DNA yield, we included an extra step to the manufacturer's instructions in which we performed a final double extraction of each sample and pooled both together. Extracted DNA was quantified using a Quant-iT Picogreen dsDNA Assay Kit (Invitrogen, Eugene, OR, USA) on a Varian Cary Eclipse fluorescence spectrometer (Agilent Technologies, Santa Clara, CA, USA). For the amplification of the markers for bacterial and archaeal V3 and V4 regions of the 16S rRNA gene and fungal ITS2 region of the rrm operon, we opted to use the primer pairs 341F (CCTAYGGGDBGCWSCAG) and 806R (GGACTACNVGGGTHCTAAT) (Frey et al., 2016) and 5.8S-Fun (AACTTTYRRCAAYGGATCWCT) and ITS4-Fun (CCTCCGCTTATTGATATGCTTAART) (Taylor et al., 2016), respectively. The samples were amplified in triplicates, pooled, and sent to the Functional Genomic Center Zürich for barcoding and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA). For more details on high throughput sequencing preparation and bioinformatic processing of microbial data, please see Supplementary Materials (Supplementary Methods and Data).

## 2.9. Root biomass

Maize root biomass was sampled in cores of 0.25 m length (0.25 m soil depth) x 0.05 m diameter (3 x 10<sup>-4</sup> m<sup>3</sup> volume) with a petrol run drill (Humax Bohrsonden, Martin Burch AG, Rothenburg,

Switzerland) at harvest time on 11.10.2017. We sampled two plants per plot, totaling 32 root samples. The soil was washed using a hydro-pneumatic elutriation system (Gillison's Variety Fabrication, Benzonia, MI, USA). The roots were separated from organic debris, sand, and other non-root material by water sieving in increasingly smaller sieves (530 to 500 μm) and then by manual separation with tweezers and by means of decantation. The clean root samples were oven dried at 55 °C for 48 h for the determination of dry weight. The root biomass data can be found in the Supplementary Material (Table S1, Supplementary Data).

## 2.10. Statistical analyses

We used linear mixed models to account for our experimental design using the lme4 package in R (Bates et al., 2015). Our field trial has a latin square design, with each cropping system treatment occurring once every column and block (for a visualization see Fig. S1). Thus, the block and column identifiers were set as fixed and random effects. Plots nested in blocks and columns was also included as random effects, accounting to the technical pseudoreplicates per plot (individual soil cores) for the models assessing the effect of cropping systems on physical variables. All other factors were treated as fixed effects. The Shapiro-Wilk test and Levene's test were employed to assess normality and homogeneity of variances, respectively. In the topsoil, with the exception of pH, all variables adhered to the assumptions of homoscedasticity. While most variables satisfied the normality assumption, certain parameters, namely macropore volume, diffusivity at 0.01 MPa and network structure indices (SP and PO), were log-transformed prior to subjecting them to linear mixed models for evaluating cropping system effects. At the subsurface level (0.4 m), all variables met the assumptions of homoscedasticity but deviated from the normality assumption. Because the data is positively skewed, for this layer, we employed generalized linear mixed models with a gamma distribution and lognormal link function to assess the influence of cropping systems on soil physical parameters. We tested whether soil texture, SOC, pH, bulk density, penetration resistance, PAW, porosity, air-filled porosity, water content, gas transport properties (Dp/D0 and log(Ka)) and network properties (SP and PO) differed among cropping systems (ANOVA). We used Tukey post hoc hypothesis test, comparing paired treatments means at α = 0.05. The effect of soil depth (i.e., 0.1 and 0.4 m) was tested separately. To account for the heteroscedasticity of pH, post-hoc pairwise comparisons were conducted using the Games-Howell test.

To explore the soil as an ecosystem for microorganisms, we calculated the α-diversity (richness) of fungal and bacterial amplicon sequence variants (ASVs) in the rhizosphere of maize. Because physical and biological samples were not taken on the exact same days and positions within each plot, we used the average values for each variable at the plot level as model input. Since our microbial and root measurements were performed in the topsoil (0.075–0.125 and 0–0.25 m respectively), we only used data at 0.1 m soil depth. First, we assessed the correlation of soil properties with biological variables and tested their significance with the package 'corrplot' (Wei and Simko, 2021). We chose the Spearman correlation coefficient to avoid assumptions of data normality (Zar, 2014). Then, in order to further explore the relationships between the physical and biological variables, while reducing the dimensionality of our dataset, we performed a partial least square regression (PLS) with the package 'pls' (Liland et al., 2021). This method works well with small data sets, dealing with multicollinearity problems or variables that deviate from normality (Scott and Crone, 2021). The PLS is also a useful method to predict dependent variables from a large set of independent variables (Lew et al., 2019). We used two forms of validation for our models: 1) model plots and 2) RMSE error estimation (Henningsson et al., 2001). In order to identify the most discriminant variables according to the models, we calculated the Variable Importance for the Projection (VIP) with the package 'plsVarSel' (Mehmood et al., 2012). The VIP estimates the importance of each explanatory variable in the model. The variables with VIP scores >1

indicate very good candidates to explain the response variable, whereas variables  $<0.8$  indicate low influence on the model (Henningson et al., 2001).

These PLS models allowed us to account separately for the variation of soil variables in relation to root biomass, microbial biomass, fungal and bacterial rhizosphere diversity as outcomes and for the differentiation among the cropping systems. This allowed us to explore which soil variables explained the most variation in soil biology, assessing soil as a habitat for roots and microorganisms. Fig. 1 provides a conceptual illustration depicting the relationship between soil properties, root growth and microbial niches. To estimate the significance of predictive performance ( $Q^2Y$ ) and variation explained by the explanatory variables ( $R^2Y$ ) at the 0.05 level, permutation tests (Szymańska et al., 2012) were performed with 200 permutations. All statistical analyses were performed in R version 4.1.0 (R Core Team, 2019).

### 3. Results

#### 3.1. Soil characteristics

The soil at the FAST trial is characterized by a loamy texture with a clay, silt and sand content of 19 %, 36 % and 44 % at 0.1 m soil depth, and 24 %, 33 % and 43 % at 0.4 m soil depth, respectively, with no significant difference among cropping systems (Supplementary Data). In the topsoil, pH was near neutral for all treatments, i.e., in the range of 6.2–7.1 (Table 1).

Soil organic carbon concentration was significantly higher at 0.1 m depth than at 0.4 m depth ( $15.75 \text{ g kg}^{-1}$  and  $6.19 \text{ g kg}^{-1}$ , respectively (F-value = 769.2,  $p < 0.001$ ). The O-IT treatment had a significantly higher soil organic carbon content at 0.1 m depth compared to conventional treatments (Table 1).

#### 3.2. Bulk density, penetration resistance and soil water retention

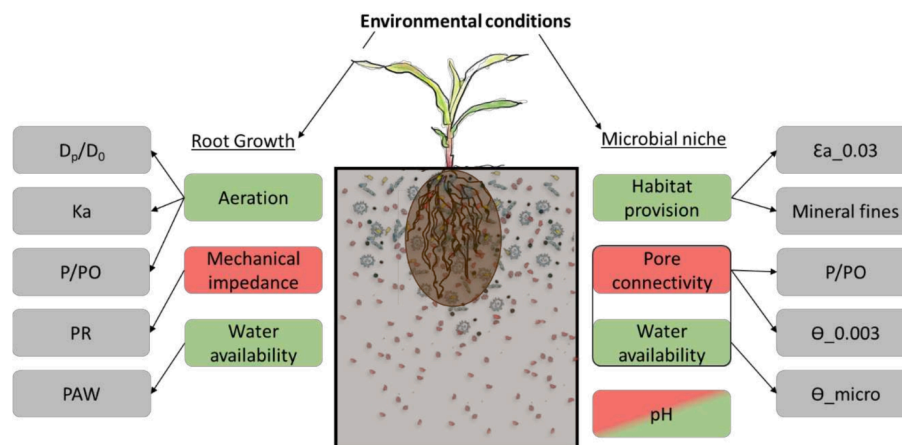
Bulk density was significantly affected by the cropping systems at

0.1 m soil depth, with lowest values for the plowed systems (C-IT, O-IT) and the highest value in the no tillage system (C-NT; Table 1). No significant differences were observed at 0.4 m soil depth. In line with bulk density, plowing decreased penetration resistance (PR) in the topsoil by 40 % ( $0.6 \text{ MPa}$ ) on average in both conventional and organic systems (C-IT, O-IT) in comparison to their respective conservation tillage treatments (C-NT, O-RT; Table 1). None of the systems was in an impeding range of mechanical resistance for plant growth. At 0.4 m soil depth, below plowing depth, penetration resistance in plowed systems reached levels above the threshold for 50 % decrease in root growth ( $2 \text{ MPa}$ ; Bengough et al., 2011), whereas root elongation conditions were less limiting under conservation tillage (Table 1).

At 0.1 m soil depth, C-IT showed the lowest and O-RT the highest (+10 %) maximum water holding capacity (WHC; Table 1). Both organic systems showed significantly higher WHC than C-IT. The water contents at different matric potentials varied among cropping systems: water contents at any given matric potential were generally lower in C-IT than those in other cropping systems (Supplementary Data). At 0.4 m soil depth, treatment effects were not statistically different (Table 1).

#### 3.3. Pore size distribution

In the topsoil (0.1 cm depth), porosity of large macropores (i.e.  $\emptyset > 100 \mu\text{m}$ ) was 32 % higher in the plowed systems (C-IT, O-IT) than those in the no-tillage and reduced tillage systems (C-NT, O-RT; Fig. 2). Air filled porosity of small macropores ( $\emptyset > 30 \mu\text{m}$ ) was 17 % higher in IT systems than in the reduced and no-tillage systems (Fig. S2). At deeper soil depths (0.4 m depth), no differences in pore volume were observed for any pore size (Fig. S3). Organic systems had higher mesoporosity than C-NT but not than those in C-IT (Fig. 2). All systems showed a pronounced stratification in macro- and mesoporosity, with higher values in the topsoil than at deeper soil depth (Fig. 2). We did not observe any differences in microporosity among the systems.

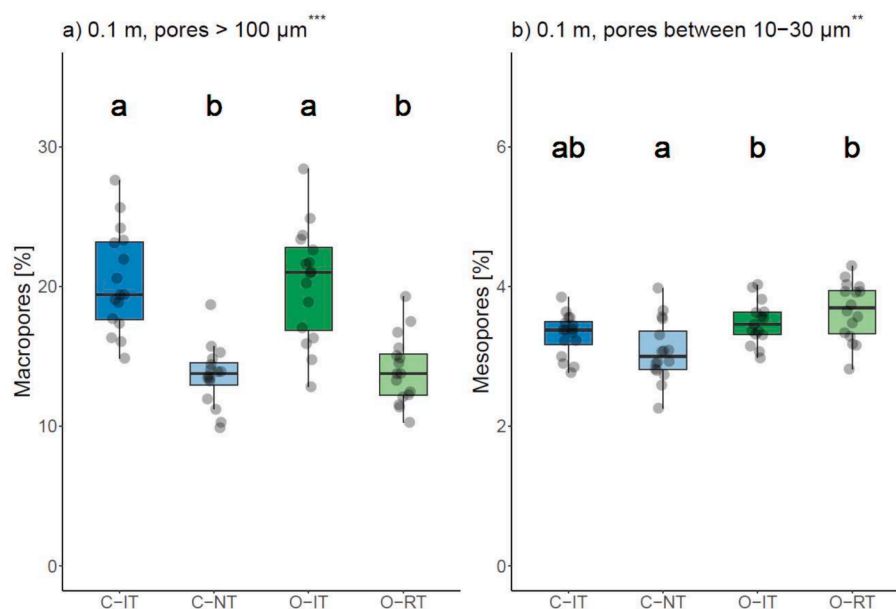


**Fig. 1.** Conceptual illustration of assessed soil properties and their hypothesized interactions with rhizosphere microbial diversity and root growth. Rhizosphere is represented as the darker area around the roots. Microbial niche refers to the fundamental environmental niche as described in Malard and Guisan (2023), the set of environmental conditions in which organisms can physiologically occur and multiply. This is a non-exhaustive list based on literature research and our measured variables. Left hand side boxes show root growth related conditions and right-hand side boxes include properties that are most likely influencing microbial niche diversity. The colored boxes represent soil processes and conditions into which the properties (grey boxes) were grouped. pH was included in the analysis as a main driver of bacterial diversity in soil (Fierer and Jackson, 2006). Green compartments are categories that potentially contribute positively (favorable conditions), while red indicates potentially negative impacts (unfavorable conditions). Rhizosphere samples for microbial analysis were collected from depths of 0.05–0.15 m, topsoil physical properties were assessed within the depth range of 0.075–0.125 m, and root sampling was conducted from 0–0.25 m. Logistical constraints, particularly related to time and resource limitations and the sampling of very different variables (e.g. soil characteristics and microbial parameters for sequencing), influenced our sampling design and this explains why not all samples were taken at exactly the same depth.  $E_a/pv$  is air-filled porosity and pore volume, and  $\theta_{micro}$  is volumetric water content at  $-0.03 \text{ MPa}$  matric potential (water held in pores  $\leq 10 \mu\text{m}$ ). SP and PO (Eq. (2) and (3)) represent macropore network characteristics derived from gas transport properties at field capacity ( $-0.01 \text{ MPa}$ ), and  $PR$  is penetration resistance.  $K_a$  is air permeability and  $D_p/D_0$  relative  $O_2$  diffusivity coefficient. Plant and microbial illustrations provided by Selma Cadot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

Mean pH, soil organic carbon content (SOC), bulk density ( $d_b$ ), penetration resistance (PR) measured in the laboratory at the matric potential of  $-0.03$  MPa, and maximum water holding capacity (WHC) for each cropping system at 0.1 m and 0.4 m soil depth. Cropping systems: C-IT conventional inversion tillage, C-NT conventional no tillage, O-IT organic inversion tillage, and O-RT organic reduced tillage. Numbers in brackets indicate standard deviation.  $F_{(df1,df2)}$  values are shown for the factor cropping system: df1: numerator degrees of freedom; df2: denominator degrees of freedom; and significance level (p) ns: non-significant; °  $p < 0.1$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ . Letters correspond to Tukey contrasts ( $\alpha = 0.05$ ) per layer and are only shown for significant contrasts.

| System          | Depth (m) | pH (H <sub>2</sub> O)    | SOC (g kg <sup>-1</sup> ) | $d_b$ (g cm <sup>-3</sup> ) | PR [MPa]                 | WHC (% Vol.)             |
|-----------------|-----------|--------------------------|---------------------------|-----------------------------|--------------------------|--------------------------|
|                 |           | n = 8                    | n = 16                    | n = 16                      | n = 8                    | n = 8                    |
| C-IT            | 0.1       | 6.2 (0.7)                | 14.8 (1.9) a              | 1.21 (0.07) ab              | 0.91 (0.57) a            | 29.8 (1.6) a             |
| C-NT            | 0.1       | 6.3 (1.1)                | 14.6 (2.8) a              | 1.37 (0.05) c               | 1.50 (0.38) b            | 31.5 (1.1) ab            |
| O-IT            | 0.1       | 6.7 (0.6)                | 16.8 (0.9) b              | 1.14 (0.1) a                | 0.88 (0.32) a            | 32.0 (1.4) b             |
| O-RT            | 0.1       | 7.1 (0.5)                | 16.8 (1.5) ab             | 1.30 (0.06) bc              | 1.47 (0.38) ab           | 32.8 (1.8) b             |
| $F_{(df1,df2)}$ |           | 1.64 <sub>(3,5)</sub> ns | 6.07 <sub>(3,12)</sub> *  | 13.83 <sub>(3,12)</sub> **  | 4.34 <sub>(3,5)</sub> °  | 6.66 <sub>(3,5)</sub> *  |
| C-IT            | 0.4       | –                        | 5.7 (2.0)                 | 1.45 (0.12)                 | 2.03 (0.95) ab           | 33.0 (4.1)               |
| C-NT            | 0.4       | –                        | 6.9 (2.7)                 | 1.51 (0.11)                 | 1.80 (0.68) b            | 31.6 (3.7)               |
| O-IT            | 0.4       | –                        | 6.5 (1.3)                 | 1.47 (0.05)                 | 2.77 (1.39) a            | 32.7 (1.4)               |
| O-RT            | 0.4       | –                        | 5.5 (2.4)                 | 1.49 (0.1)                  | 1.82 (0.61) b            | 31.7 (4.1)               |
| $F_{(df1,df2)}$ |           | –                        | 1.06 <sub>(3,12)</sub> ns | 0.43 <sub>(3,12)</sub> ns   | 10.08 <sub>(3,5)</sub> * | 0.34 <sub>(3,5)</sub> ns |



**Fig. 2.** Box plots illustrating the distribution of a) Macroporosity (large macropores,  $>100 \mu\text{m}$ ) and b) mesoporosity (pores between 10 and  $30 \mu\text{m}$ ) across the different cropping systems. Each box plot represents the median (line inside the box), interquartile range (box), and range of the data (whiskers). The bottom and top borders of the box correspond to Q1 (the first quartile) and Q3 (the third quartile), respectively. Outliers beyond the whiskers are plotted individually. The panels refer to sampling at 0.1 m soil depth, results for 0.4 m can be found in supplementary material (Fig. S3). Dots represent individual soil core samples, i.e., four cores per cropping system plot. Cropping systems: C-IT conventional inversion tillage, C-NT conventional no tillage, O-IT organic inversion tillage, and O-RT organic reduced tillage. Significance levels for the factor cropping system ( $\alpha = 0.05$ ): ns: non-significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ . Letters represent Tukey contrasts ( $\alpha = 0.05$ ).

### 3.4. Gas transport properties and soil pore network indices

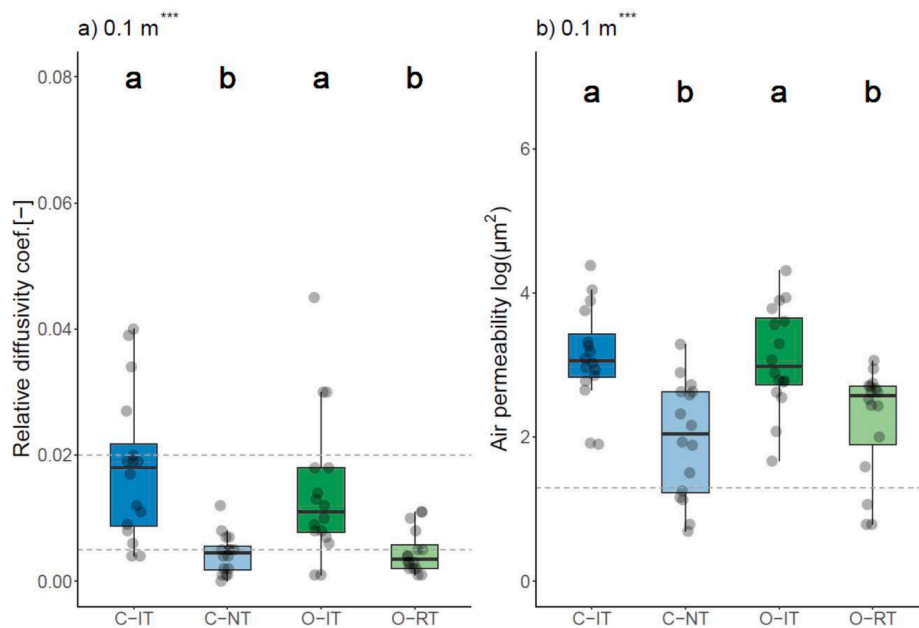
We observed increased values of gas transport properties ( $K_a$  and  $D_p/D_0$ ) in the topsoil (0.1 m soil depth) of the plowed treatments (C-IT, O-IT; Fig. 3). At the matric potential of  $-0.03$  MPa, all cropping systems had air permeability values above the critical thresholds for root growth ( $\log(K_a) < 1.3$ ; Fish and Koppi, 1994) and for minimum  $O_2$  diffusivity needed to meet high  $O_2$  demands ( $D_p/D_0 = 0.02$ ) (Schjøning et al., 2003) (Fig. S4). However, at field capacity ( $-0.01$  MPa) and under moist conditions ( $-0.003$  MPa), conservation tillage – both no-till and reduced tillage – were not sufficiently aerated to meet high  $O_2$  demands (Fig. 3 and Fig. S5 respectively). At 0.4 m soil depth, air permeability at field capacity was below the critical threshold for root growth for all cropping systems (Fig. S5), and the  $O_2$  diffusivity coefficient was below the  $O_2$  consumption threshold (Schjøning et al., 2003), but above the critical

level for low  $O_2$  demands (Stepniewski, 1980). At  $-0.003$  MPa, air permeability in 0.4 m soil depth of O-IT was lower than in their conventional counterpart (C-IT; Fig. S6).

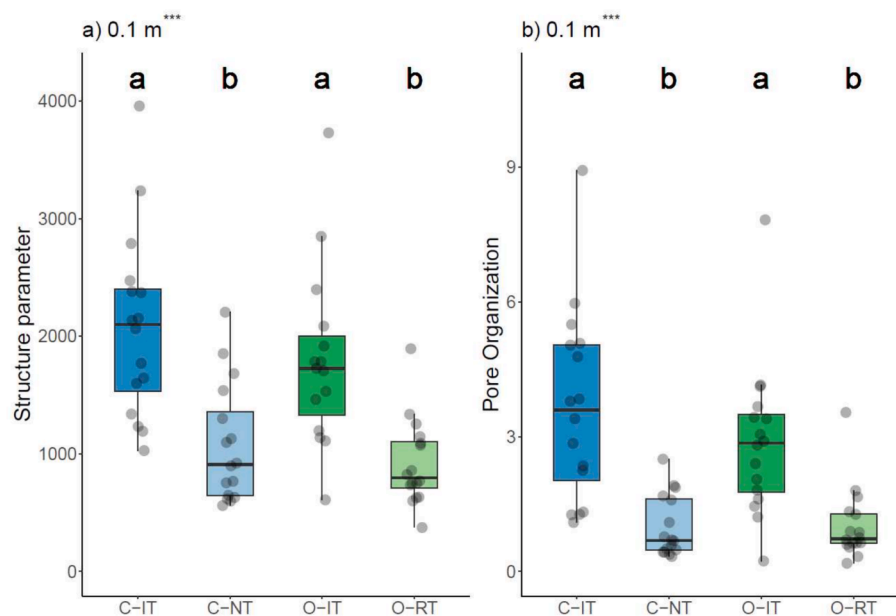
Inversion tillage in C-IT and O-IT increased the pore structure indices SP and PO (Fig. 4), indicating higher pore connectivity or lower tortuosity in the topsoil (0.1 m depth). In the deeper soil layer (0.4 m depth), variability was high, and no statistical differences were found among cropping systems (Fig. S7).

### 3.5. Soil physical environment for roots and microorganisms

Twelve of the 28 investigated soil variables at 0.1 m soil depth were significantly correlated with at least one of the biological variables (Table S3). Root biomass was positively correlated with pore architecture (SP), silt content, and air-filled porosity ( $E_a$  [% vol.]) at  $-0.003$



**Fig. 3.** Box plots illustrating gas transport properties at field capacity (matric potential of  $-0.01$  MPa) across cropping systems. Panel a) shows relative gas diffusivity coefficient ( $D_p/D_0$ ), panel b) air permeability ( $\log K_a$ ). Each box plot represents the median (line inside the box), interquartile range (box), and range of the data (whiskers). The bottom and top borders of the box correspond to Q1 (the first quartile) and Q3 (the third quartile), respectively. Outliers beyond the whiskers are plotted individually. The panels refer to sampling at 0.1 m soil depth, results for 0.4 m can be found in supplementary material (Fig. S5). Dashed lines are critical values for minimum  $O_2$  diffusivity needed to meet high ( $D_p/D_0 = 0.02$ ) (Schjønning et al., 2003) or low ( $D_p/D_0 = 0.005$ ) (Stepniewski, 1980)  $O_2$  demands and impeding root growth ( $\log K_a < 1.3$ ; Fish and Koppi, 1994). Dots represent individual soil core samples, i.e., four cores per cropping system plot. Cropping systems: C-IT conventional inversion tillage, C-NT conventional no tillage, O-IT organic inversion tillage, and O-RT organic reduced tillage. Significance levels for the factor cropping system ( $\alpha = 0.05$ ): ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Letters represent Tukey contrasts ( $\alpha = 0.05$ ).



**Fig. 4.** Box plots illustrating pore connectivity or tortuosity derived from gas transport properties across cropping systems. Panel a) shows the structure parameter (SP) and panel b) the pore organization (PO) indices at field capacity (matric potential of  $-0.01$  MPa). Each box plot represents the median (line inside the box), interquartile range (box), and range of the data (whiskers). The bottom and top borders of the box correspond to Q1 (the first quartile) and Q3 (the third quartile), respectively. Outliers beyond the whiskers are plotted individually. The panels refer to sampling at 0.1 m soil depth, results for 0.4 m can be found in supplementary material (Fig. S7). Dots represent individual soil core samples, i.e., four cores per cropping system plot. Cropping systems: C-IT conventional inversion tillage, C-NT conventional no tillage, O-IT organic inversion tillage, and O-RT organic reduced tillage. Significance levels for the factor cropping system ( $\alpha = 0.05$ ): ns: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Letters represent Tukey contrasts ( $\alpha = 0.05$ ).

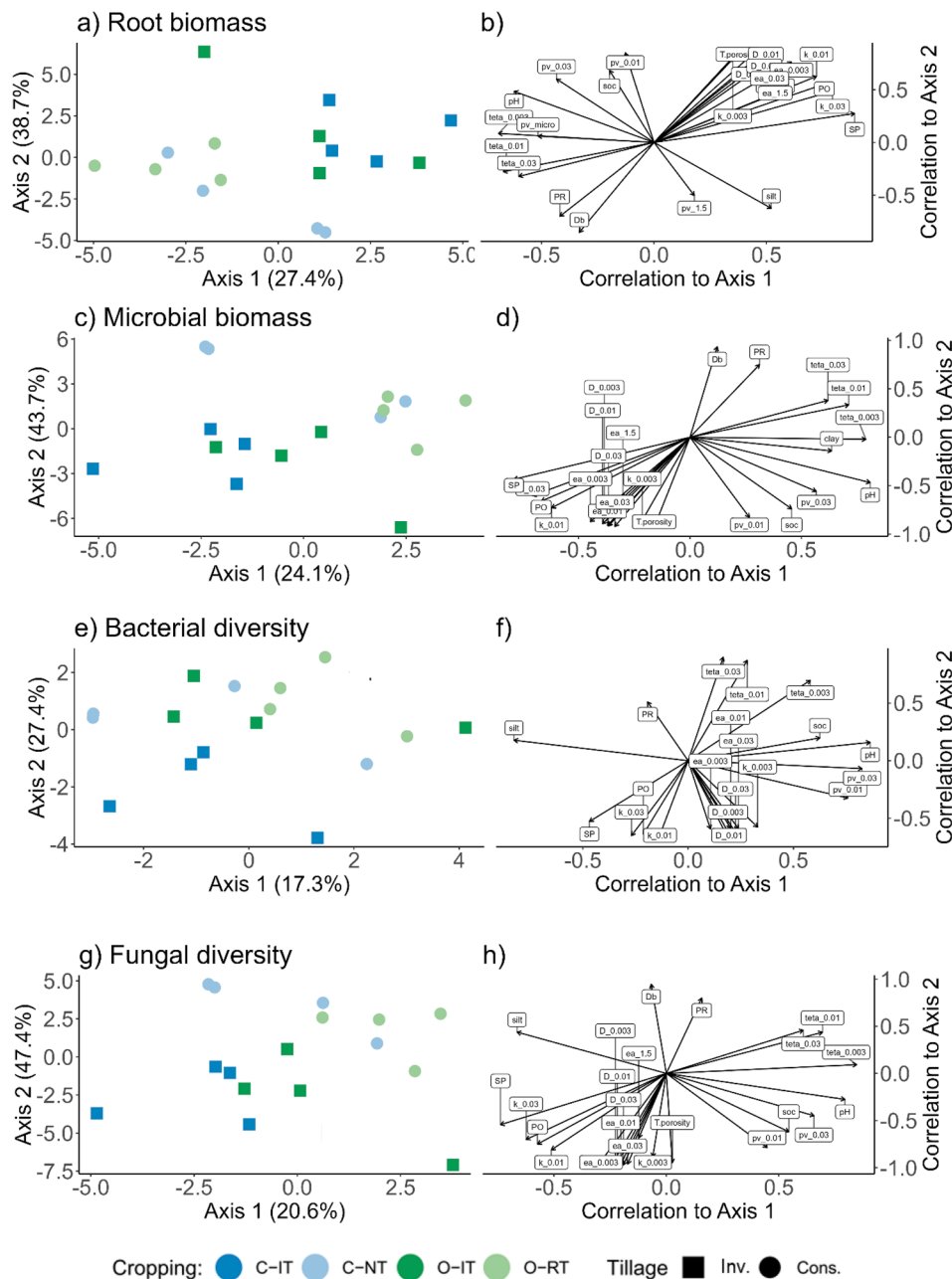
MPa (large macropores) and  $-1.5$  MPa, but negatively correlated to mesoporosity. Microbial biomass was significantly correlated to eight of the identified soil physical variables. Pore organization (PO), structural

parameter (SP), and air permeability at  $-0.03$  MPa were negatively correlated to microbial biomass, whereas pH, clay content, soil water content at  $-0.003$  and  $-0.001$  MPa were positively correlated to

microbial biomass. All microbial variables, i.e., biomass and diversity (i.e., species richness in the rhizosphere of maize), were negatively correlated to silt content and positively correlated to pH, mesoporosity and water content at  $-0.003$  MPa, which were opposite trends to root biomass. Although rhizosphere fungal and bacterial richness were similarly correlated to the soil variables, the strength of correlation was different for both groups. Bacterial diversity was more strongly correlated to clay content and mesoporosity than fungal diversity. On the other hand, fungal diversity was more strongly correlated to soil water content at  $-0.003$  and  $-0.01$  MPa than bacterial diversity. With the exception of microbial biomass, which was higher in O-RT followed by

C-NT, we did not observe any statistical difference in biological variables among cropping systems (Table S2).

The variable importance for projection (VIP) of the partial least square regression indicates that, pH, silt content, pore network indices (SP and PO), porosity of small macropores and mesopores, air permeability at  $-0.03$  MPa and water content at  $-0.003$ ,  $-0.01$  and  $-0.03$  MPa were good descriptors for all biological components (VIP scores  $>0.8$  for root biomass, microbial biomass, rhizosphere bacterial and fungal diversity; Table S3). All variables identified as highly correlated to the biological parameters by the Spearman correlations (i.e., significant correlations above 0.4) were supported by the VIP analysis.



**Fig. 5.** PLS score plots (a, c, e and g) and variable correlation plots (b, d, f and h) for root biomass, microbial biomass, bacterial diversity, and fungal diversity as response variables. Only soil variables significantly correlated ( $p < 0.05$ ) and highly contributing to the variation explained ( $-0.5 < \text{or} > 0.5$ ) are displayed in the correlation plots. Axis 1 depicts the predictive and axis 2 the orthogonal variation derived from our analysis. The predictive variation accounts for the proportion of data variance directly associated with the predictor variables. Conversely, the orthogonal variation captures variability unrelated to these predictors but still inherent in the dataset. For more details on variable abbreviations, please see caption of Fig. 1 or supplementary data. Cropping systems: C-IT conventional inversion tillage, C-NT conventional no tillage, O-IT organic inversion tillage, and O-RT organic reduced tillage. Tillage: Inv. inversion tillage (IT) and Cons. conservation tillage (NT and RT).



However, many variables identified as important descriptors by the PLS models ( $VIP > 0.8$ ) were not necessarily significantly linearly correlated (Table S3).

The most important predictors for root biomass were tortuosity or connectivity of the pore network (SP), air-filled porosity at  $-1.5$  MPa, mesoporosity and water content at  $-0.003$  MPa ( $\theta_{0.003}$ ) (Table S3). Microbial biomass was also influenced by the pore network characteristics, with the four highest VIPs being the tortuosity or connectivity of the pore network (SP), clay content, pH and mesoporosity. The four most important predictors of bacterial diversity were silt content, pH and porosity of small macropores and mesopores (i.e.,  $pv_{0.003-0.01}$  and  $pv_{0.01-0.03}$ ). Highest VIPs for fungal diversity were similar to those influencing bacterial diversity, with the exception of water content at  $-0.003$  MPa ( $\theta_{0.003}$ ) playing a stronger role than the air-filled porosity at field capacity ( $-0.01$  MPa).

Generally, the inversion tillage plots (C-IT, O-IT) were characterized by higher aeration and pore network metrics, such as gas transport variables, SP and PO and air-filled porosity at  $-0.003$  MPa ( $Ea_{0.003}$ ; Fig. 5). Soil properties that were positively related to the microbial community were lower in these plowed systems, i.e., porosity of small macropores and mesoporosity, as well as water contents ( $\theta$ ) at  $-0.003$ ,  $-0.01$  and  $-0.03$  MPa. The higher water content at all three matric potentials in organic systems strongly influenced changes in the bacterial diversity, leading to a differentiation between O-IT and C-IT plots (Fig. 5).

It is noticeable that two of the C-NT plots diverged from the other C-NT and O-RT plots in most of the PLS scores ordination plots. This segregation was largely influenced by silt content, pH, and bulk density. The soil in these two C-NT plots had higher silt content, was more acidic, showed higher bulk density, and was, in general, less associated to features that were positively correlated to microbial parameters, such as porosity and water content at all matric potentials.

When examining the differentiation of groups in our PLS score plots (Fig. 5), we observed distinct separation among cropping systems based on tillage (inversion vs. conservation), management (conventional vs. organic), and their combinations (C-IT, C-NT, O-IT, O-RT). Tillage exhibited the highest degree of explanation based on the measured soil variables, followed by management type and cropping systems (Table S). Note that the error estimations (RMSE) for the factorial variables is non-informative, since they are categorical. The PLS score ordinations and variables correlation plots for the factorial variables can be found in the Supplementary Material (Figs. S8-S10).

The PLS models could explain the variation in the biological dataset relatively well based on the assessed soil variables described in Table S4. The cumulative  $R^2$  of each response variables, i.e.,  $R^2Y(\text{cum})$ , represents the proportion of variation in each of the response variables explained by the soil variables included in the respective model (Table S4). The soil variables were good predictors for microbial biomass and bacterial diversity ( $R^2Y = 0.82$  and  $R^2Y_{\text{Bac}} = 0.74$ , respectively) and less effective for fungal diversity and root biomass ( $R^2Y = 0.64$  for both groups, Table S4).

## 4. Discussion

### 4.1. Impact of different management practices on soil structure

This study demonstrated that both soil tillage, management system (organic vs conventional) and their interactions influence soil physical properties. Irrespective of the management strategy (conventional or organic), inversion tillage increased macroporosity and macropore connectivity or continuity in the topsoil, in line with previous studies (Dal Ferro et al., 2014; Lucas et al., 2019; Lucas et al., 2020; Pires et al., 2020). Continuous pore networks and fissures could decrease mechanical impedance by preferential root growth (Bengough et al., 2011; Colombi et al., 2017; Kuzyakov and Blagodatskaya, 2015; Landl et al., 2017). This was reflected in an increased gas transport capacity and

lower penetration resistance in ploughed plots (C-IT, O-IT). From an agronomic perspective, these are positive features, and under favorable climatic and nutrient conditions, crop yields tend to be higher in tilled systems (Pittelkow et al., 2015b), as also found in the FAST trial for the summer crops maize, field bean and pea-barley mixture (Wittwer et al., 2023), but less for winter wheat. In our study, conservation tillage practices and organic management enhanced the retention of water in the soil profile, which was also observed under different crops (pea-barley mixture; Sun et al., 2021). This suggests that soil moisture would not be so easily lost under drought events or heatwaves, when evapotranspirative demand is increased (Dai et al., 2018), which could improve the system's resilience to drought under conservation tillage practices and organic management (García-Tejero et al., 2020). However, drought reduced yields of three crops grown in the four cropping systems in the FAST experiment (Wittwer et al., 2023; Sun et al., 2024), suggesting a limited capability of organic farming and conservation tillage to enhance agroecosystem resilience to severe drought, at least in the context of this experiment (i.e., without cover crops and full nitrogen fertilization). Moreover, system resilience will also depend on the crop grown and if soil water is indeed available to plant roots. In a complementary study (Sun et al., 2021), we found no differences in drought stress among cropping systems for pea, but barley plants were most stressed under C-NT and least stressed under O-IT. The tendency of the organic plowed system (O-IT) having higher water holding capacity could be related to 1) a slightly higher SOC in these systems, or 2) the effects of pore wall coating on water retention, mediated by the increased stability of aggregates (Fér et al., 2020). The SOC concentrations at this study should be taken with caution, due to the fact that the intact soil cores omitted the soil organic carbon-enriched top few centimeters (surface layers) in the no-tillage and reduced tillage systems. To explore whether specific soil pore properties within systems influence water holding capacity, more detailed studies using non-destructive technologies, such as X-ray micro computed tomography would be necessary.

### 4.2. Soil environment affecting root biomass and microbial diversity

Based on PLS models, we observed that the cropping system management clearly altered soil properties, creating environments correlated with either favorable or detrimental conditions for root biomass or microbial biomass and rhizosphere diversity. Particularly, the biological parameters we assessed showed stronger relationships with aeration in soils under inversion tillage (IT) and water retention capacity under conservation tillage practices (RT, NT). It has been shown earlier that aeration and water are very important factors determining root development (Khalil et al., 2020), and in fact, these were the variables contributing most significantly to the differentiation between tillage treatments in terms of root growth. Overall, inversion tillage was related to a facilitative soil environment for root growth by decreasing penetration resistance and increasing gas transport and soil porosity in the topsoil.

While earlier research has proposed that a majority of soil bacteria thrive in micropores ranging from  $0.8$  to  $30$   $\mu\text{m}$ , particularly within microaggregates (Ranjard et al., 2000; Ranjard and Richaume, 2001; Sessitsch et al., 2001; Torsvik and Øvreås, 2002; Wilpiseski et al., 2019), our observations did not reveal an association between either microbial biomass or rhizosphere microbial richness with pores below  $10$   $\mu\text{m}$  in diameter. This finding aligns with other studies, that indicate an association between the flow of nutrients, gases, and organisms with pores below  $30$   $\mu\text{m}$  (Xia et al., 2022). Our Partial Least Squares (PLS) models exhibited greater robustness in describing microbial biomass and bacterial richness compared to fungal richness, and demonstrated stronger correlations of microbial diversity with pores in the range of  $10$ – $100$   $\mu\text{m}$ . A growing body of literature highlights the significance of pores within the  $30$ – $150$   $\mu\text{m}$  range in influencing essential soil functions such as carbon decomposition (Kravchenko and Guber, 2017;

Kravchenko et al., 2019), N<sub>2</sub>O emissions (Kim et al., 2022), and providing suitable microbial habitats (Kravchenko and Guber, 2017). Because they also serve as preferential sites for fine root colonization and retain water for more extended periods compared to larger pores, pores within this range strike a balance between nutrient and water supply and optimal size to organism colonization, offering in addition protection from predators (Kravchenko et al., 2019).

Furthermore, we found that the microbial biomass as well as the diversity of fungi and bacteria in the rhizosphere were indeed correlated with soil textural variables such as clay and silt content, supporting earlier studies (Sessitsch et al., 2001). We did not observe differences in porosity below 10 µm among systems; however, mesoporosity was highest in organic systems and C-IT, whereas C-NT had the lowest values. This shows that no-till is not a single bullet solution for maintaining soil quality, regarding microbial diversity. A more comprehensive evaluation over a longer-term study period is warranted, as the effects of no-till practices may be time-dependent. Notable improvements are typically observed in no-till systems with a minimum of 15 years after implementation (Kay and VandenBygaart, 2002). Thus, our assessment after 8 years may not sufficiently capture the soil network's restructuring. The loss of microbial habitat diversity in C-NT in comparison to O-RT highlights the importance of organic inputs and soil decompaction efforts into creating favorable microbial soil environments (Longepierre et al., 2021).

We also observed that soil water holding capacity was positively correlated with all microbial metrics. Bacteria are found in the soil in a patchy distribution (Kuz'yakov and Blagodatskaya, 2015), and move either passively by transport in water (Dechesne et al., 2010) and soil organisms (Yang and van Elsas, 2018), actively by developing motile structures such as flagella (Ebrahimi and Or, 2015) or even through sensing electric fields (Chong et al., 2021). The passive transport forms are intimately related to water films (Tecon and Or, 2017). The relationship between soil water content and bacterial diversity, however, is not linear. A growing body of literature has suggested that connectivity of water-filled pores, promoted by increased degree of saturation in the soil, decreases bacterial diversity (Bickel and Or, 2020; Carson et al., 2010; Tecon and Or, 2017). However, if the unconnected patches are too small (i.e., drier soil condition), species could start to interact negatively, for example, by increasing competitive exclusion (Ratzke et al., 2020; Tecon et al., 2018). Our results support the importance of soil water retention for the microbial community; however it does not support the hypothesis that greater connectivity due to saturation leads to lower microbial diversity. Air-filled porosity, which could indicate a higher disconnection of the water film, was actually more correlated to root and microbial biomass than microbial diversity. In fact, the richness of microorganisms was positively correlated with water content at -0.01 MPa (i.e. when macropores were air-filled) and not responsive to water content at -0.03 MPa when macro- and mesopores were air-filled. A similar pattern was observed in a microcosm experiment which suggests that this response could be a result of the higher flow of C and nutrients in macropores, allowing for different microbes to react on substrates, therefore increasing the co-existence of different taxa (Xia et al., 2022).

Air permeability and metrics of pore network architecture (P and PO) were negatively associated with microbial biomass and diversity. This negative relationship could in fact reflect the impacts of tillage on fungal communities, considering that aeration and soil pore network metrics were higher in inversion tillage systems. The production and maintenance of a stable hyphae network is related to a stable soil pore architecture (Pot et al., 2022) and could be easily disrupted by tillage (Kabir, 2005). This means that although ploughing was positively related to root biomass, it has the potential to hinder soil communities, especially fungi, and its negative effects were not buffered by the addition of organic inputs.

The design of sustainable cropping systems is a complex task that needs to take in consideration the growing food demands and the

maintenance of a heterogeneous soil habitat. This means fine tuning the positive contributions of each practice, such as for example the positive decompaction effects of tillage, when carefully planned, promoting soil aeration and water fluxes in the topsoil, the positive impacts of organic matter addition in organic farming, or the increased water retention promoted by conservation agriculture practices.

#### 4.3. Further needs of research

Our study demonstrated the impact of tillage and management system on a range of soil parameters. The results were based on one specific experiment and further studies need compare a broader number of fields to obtain results that can be better generalized. Moreover, since our analyses were based on correlations, we cannot establish causal relationships. Nevertheless, our results provided empirical evidence that microbial biomass and rhizosphere microbial richness are differentially affected by soil properties. The high error estimations (RMSE > 0.5) and low goodness of prediction values (Q<sup>2</sup>) indicated the presence of significant variables that have yet to be accounted for. For example, recent studies suggest the importance of pore size heterogeneity in influencing the microbial community composition (Xia et al., 2022) and soil functioning (Kravchenko et al., 2019; Negassa et al., 2015; Quigley et al., 2018). The p values pR<sup>2</sup>Y and pQ<sup>2</sup> were not significant for root and fungal models which means that although root and fungal variability were well explained by the models, they were not statistically significant at the α = 0.05 level and results should be taken with care. The inclusion of other important variables, exclusion of less relevant ones to avoid overfitting, or a higher sampling effort could change this picture.

Soil pore architecture metrics, e.g., pore connectivity, topology and biopore metrics (i.e., pores created by bioturbation, such as root growth or earthworm movements in the soil), as well as pedo-climatic factors such as temperature and nutrient contents might have an effect on soil communities. With the inclusion of such environmental variables, the existing uncertainty might be reduced. Similarly, accounting for the impact of crop phenology and pedological cycles in agroecosystems (e.g. seasonal compaction after tillage) might be relevant to explain differences in microbial diversity, since demands for plant growth and thus competition with microbes and microbial dynamics are not static over the growing season.

Furthermore, our analyses were limited to the topsoil layer, neglecting potential influences stemming from increased compaction in the subsoil under plowed systems, which could hinder root penetration at deeper depths. Logistical constraints, notably related to time and resource limitations, influenced our sampling design. The collection and processing of root samples proved to be labor-intensive, while the sequencing of soil microorganisms in both soil layers posed cost challenges. Therefore, we focused on the topsoil due to its pivotal role in harboring active biological and physicochemical processes essential for plant development and nutrient cycling. Although significant correlations were observed within the sampled depths for microbial communities, soil properties, and root biomass in the topsoil, it is crucial to ensure that these associations persist when sampling depths fully align with root and microbial biomass. Examining a wider range of soil types and layers, encompassing both surface and subsoil strata, will contribute to a more holistic understanding of the complex interplay among cropping system practices, soil compaction, and their collective impacts on soil physical properties, root growth, and rhizosphere microbial diversity.

## 5. Conclusions

We assessed the impact of four cropping systems on a range of soil physical properties related to water retention, penetration resistance, gas transport, and pore network characteristics in a long-term field trial. As hypothesized, inversion tillage created a facilitative soil environment for root growth by decreasing penetration resistance and increasing gas

transport and soil porosity. Conservation tillage and organic management (regardless of tillage system) increased water holding capacity, however, C-NT systems had soil characteristics that were unfavorable for the creation of microbial habitats, such as higher bulk density and lower mesoporosity. This trade-off between facilitating root growth while reducing water retention creates a challenge for developing climate-smart cropping management practices, which should balance benefits and disadvantages of any particular soil management practice in arable farming. Enhancing soil functions related to microbial habitat, carbon sequestration, nutrient cycling, and soil water supply for plant use need to be considered for climate adaptation via enhancing agroecosystems resilience to climate change.

#### CRedit authorship contribution statement

**Emily M. Oliveira:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Raphaël Wittwer:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Martin Hartmann:** Writing – review & editing, Supervision. **Thomas Keller:** Writing – review & editing, Supervision, Conceptualization. **Nina Buchmann:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marcel G.A. van der Heijden:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

#### Declaration of competing interest

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

#### Data availability

Data will be made available on request.

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#### Author contributions

EOH: Collecting, processing and analyses of the soil samples, data processing and analysis, writing the manuscript. RW: Managing the field experiment, collecting soil samples, contributing to data analysis and discussion, writing and revising the manuscript. MH: Supervising the soil microbial diversity analyses, scientific discussions, revising the manuscript. NB: Funding acquisition, scientific discussions, revising the manuscript. TK: Supervising the soil physical analyses, scientific discussions, revising the manuscript. MvdH: Funding acquisition, initiation of the FAST experiment, scientific discussions, revising the manuscript.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2024.116927>.

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