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RESEARCH ARTICLE

Soil chemical and microbial gradients determine accumulation of root-exuded secondary metabolites and plant–soil feedbacks in the field

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Abstract

Introduction: Harnessing positive plant–soil feedbacks via crop rotations is a promising strategy for sustainable agriculture. These feedbacks are often context-dependent, and how soil heterogeneity explains this variation is unknown. Plants influence soil properties, including microbes, by exuding specialized metabolites. Benzoxazinoids, specialized metabolites released by cereals such as wheat and maize, can alter rhizosphere microbiota and performance of plants subsequently growing in the exposed soils and are thus an excellent model to study agriculturally relevant plant–soil feedbacks.

Materials and Methods: To understand local variation in soil properties on benzoxazinoid-mediated plant–soil feedbacks, we conditioned plots with wild-type maize and benzoxazinoid-deficient *bx1* mutants in a grid pattern across a field, and we then grew winter wheat in the following season. We determined accumulation of benzoxazinoids, root-associated microbial communities, abiotic soil properties and wheat performance in each plot and then assessed their associations.

Results: We detected a marked gradient in soil chemistry and microbiota across the field. This gradient resulted in significant differences in benzoxazinoid accumulation, which were explained by differential benzoxazinoid degradation rather than exudation. Benzoxazinoid exudation modulated microbial diversity in root and rhizospheres during maize growth, but not during subsequent wheat growth, while the chemical fingerprint

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of benzoxazinoids persisted. Averaged across the field, we did not detect feedbacks on wheat performance and defence, apart from a transient decrease in biomass during vegetative growth. Closer analysis, however, revealed significant feedbacks along the chemical and microbial gradient of the field, with effects gradually changing from negative to positive along the gradient.

Conclusion: Overall, this study revealed that plant–soil feedbacks differ in strength and direction within a field and that this variation can be explained by standing chemical and microbial gradients. Understanding within-field soil heterogeneity is crucial for the future exploitation of plant–soil feedbacks in sustainable precision agriculture.

KEYWORDS

crop rotation, environmental gradient, maize, plant–microbe interactions, plant–soil feedback, secondary metabolites, soil chemistry, wheat

1 | INTRODUCTION

Plants influence the soil they grow in, which in turn influences the performance of subsequent plants. In crop production, designing a suitable crop rotation makes use of positive plant–soil feedbacks (van der Putten et al., 2013). Identifying and exploiting the mechanisms of plant–soil feedbacks in crop rotations has been proposed as a promising tool to promote sustainable agriculture by leveraging agroecological effects (Mariotte et al., 2018). A key challenge in this context is the spatial and temporal variability of plant–soil feedbacks in the field (Smith-Ramesh & Reynolds, 2017).

Plant–soil feedbacks are attributed to a number of different mechanisms, including changes in mutualist and pathogen abundance, microbiota composition, nutrient availability, and other soil chemical properties (Bennett & Klironomos, 2019; Bever et al., 2012; Pineda et al., 2020). These drivers can affect germination, plant performance (van der Putten et al., 2013; Tawaha & Turk, 2003), as well as pathogen and herbivore resistance (Kos et al., 2015b; Ma et al., 2017; Pineda et al., 2020). Given that all these factors are highly heterogeneous, one can expect strong context dependency and spatiotemporal variation in the resulting feedback effects.

Plant-associated microbial communities have gained attention as drivers of plant–soil feedbacks in the past (Bever et al., 2012). An important mechanism in how plants shape their microbiota is through root exudates, defined as the secretion of primary and secondary metabolites to the surrounding soil (Pang et al., 2021). Prominent examples of secondary metabolites involved in structuring root or rhizosphere microbiotas are benzoxazinoids, coumarins, flavones, and triterpenes (Hu, Robert, et al., 2018; Huang et al., 2019; Stringlis et al., 2019; Voges et al., 2019; Yu et al., 2021). Changes in root-associated microbiotas can in turn increase plant growth and defence (Berendsen et al., 2012; Pieterse et al., 2014).

Spatial and temporal variation in plant–soil feedbacks has been studied mostly for soil nutrients, temperature (De Long et al., 2019; Smith-Ramesh & Reynolds, 2017), drought (Fry et al., 2018) and the interaction of abiotic factors with soil biota (De Long et al., 2019; Kaisermann et al., 2017). Further, local soil biotic communities represent key determinants of plant–soil feedbacks across time and space (Bennett et al., 2017; Revillini et al., 2016). How local (i.e., within-field) variation in soil parameters affects plant–soil feedbacks driven by exuded root metabolites are largely unknown. Furthermore, the dynamic interplay between exuded metabolites and their degradation or metabolization by rhizosphere microbial communities can be expected to add to the variation in plant–soil feedbacks.

Benzoxazinoids, a class of secondary metabolites common in grasses, including maize and wheat, have been shown to be bioactive in many ways (Niemeyer, 2009). They are long known to be involved in allelopathy and defence against insects and pathogens (Niemeyer, 2009; Schandry & Becker, 2020). More recently, they have been shown to alleviate plant growth-suppressive effects provoked by preceding plants (Gfeller, Thönen, et al., 2023). They can also chelate iron and aluminium (Hu, Mateo, et al., 2018; Zhao et al., 2019; Zhou et al., 2018). In the past few years, benzoxazinoids have repeatedly been shown to shape root-associated microbiotas (Cadot, Guan, et al., 2021; Cotton et al., 2019; Hu, Robert, et al., 2018; Kudjordjie et al., 2019). Maize roots predominantly excrete DIMBOA-Glc, HDMBOA-Glc and DIMBOA, and these benzoxazinoid compounds are rapidly converted into MBOA in soil (see Supporting Information: Figure S1 for full names and chemical structures; Hu, Robert, et al., 2018). Ultimately, soil microorganisms can further metabolize MBOA (half-life: days to weeks) to AMPO (half-life: weeks to months) and thereby alter their availability in soils (Etzerodt et al., 2008; Macías et al., 2004). The exudation of benzoxazinoids into the soil can feedback on plant performance of the next plant grown in that soil. These so-called benzoxazinoid-dependent plant–soil feedbacks have so far been reported for maize–maize and maize–wheat cropping sequences in the greenhouse (Cadot, Gfeller, et al., 2021; Hu, Robert, et al., 2018) and in the field (Gfeller, Waelchli, et al., 2023). Soil chemistry was relatively homogenous throughout the field of Gfeller, Waelchli et al. (2023) and benzoxazinoid exudation by maize resulted in an increase in wheat yield. If and how such

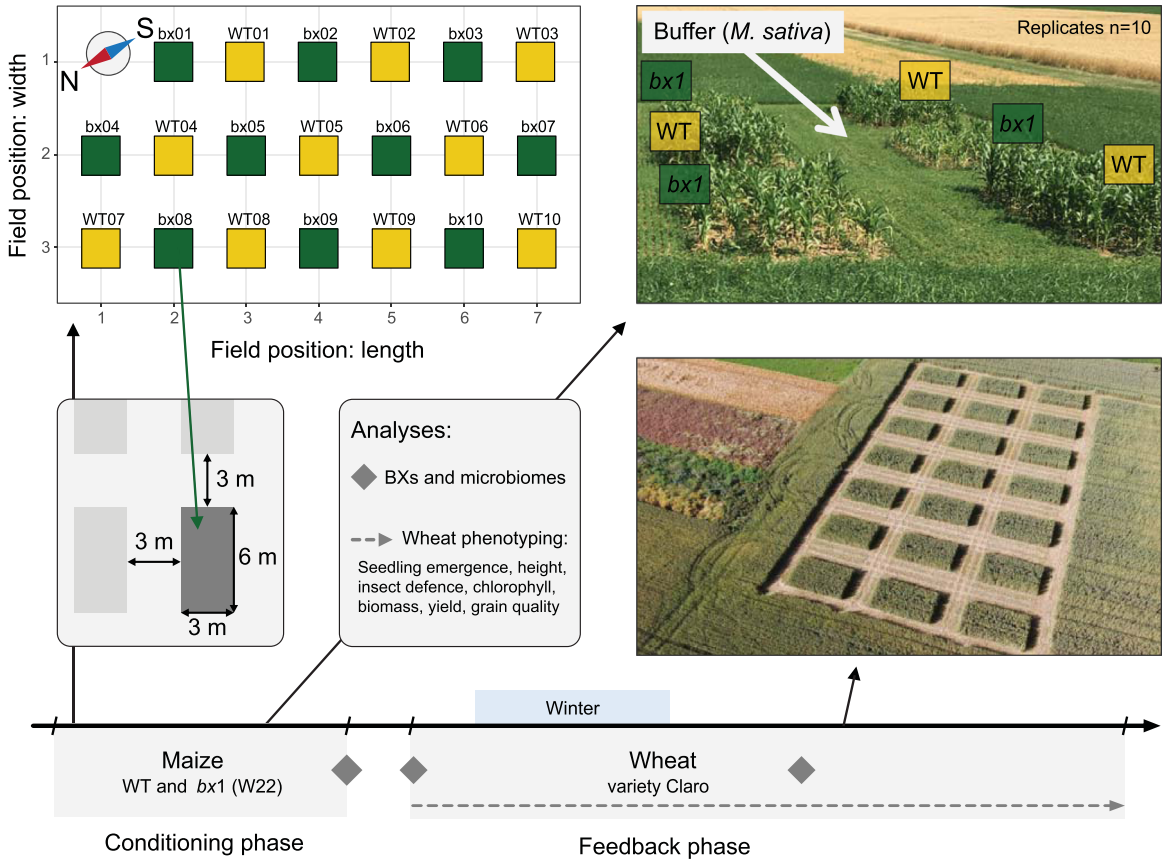

FIGURE 1 Experimental set-up. To examine the effect of maize benzoxazinoid soil conditioning on subsequent wheat growth, defence, yield and grain quality, we conducted a 2-year field experiment in Changins, Switzerland. First, wild-type (WT) and benzoxazinoid-deficient *bx1* mutant plants of the maize line W22 were grown on 10 plots each (plot dimensions 3 m × 6 m). As a buffer, *Medicago sativa* was grown between maize plots. After maize harvest, the winter wheat variety Claro was sown. Wheat growth and defence were intensively phenotyped throughout cultivation. At harvest, yield was determined and grain quality was analysed. Soil benzoxazinoid concentrations and microbiotas were analysed at maize harvest, wheat sowing and wheat growth. For microbiota analysis, roots, rhizospheres and the soils surrounding the plants were sampled, except for the time point at wheat sowing, where only soil was present. For more details, please refer to Section 2.

feedbacks act under heterogeneous chemical soil conditions is largely unknown.

In this study, we investigated in a maize–wheat crop rotation how soil heterogeneity influences benzoxazinoid-mediated plant–soil feedbacks. We set up a 2-year field experiment where we first grew maize to condition the soil followed by winter wheat to score the feedbacks (Figure 1). The soil was conditioned either by benzoxazinoid-producing wild-type maize or by benzoxazinoid-deficient *bx1* mutant plants, and we then assessed feedbacks in 20 replicate plots within the field. Effects of maize benzoxazinoid soil conditioning on wheat performance were then analysed, taking the gradient in soil chemistry present in the field into account. Detailed measurements of benzoxazinoid accumulation and changes in soil microbiota were used to determine to what extent these factors interact with soil heterogeneity to explain the observed variation in plant–soil feedbacks. Overall, our results show a high context dependency of secondary metabolite-mediated plant–soil feedbacks. Understanding such context dependencies is crucial to successfully employ the concept of plant–soil feedbacks in crop rotations.

2 | MATERIALS AND METHODS

2.1 | Plant material

Wild-type maize (*Zea mays*) and the corresponding benzoxazinoid-deficient *bx1* transposon insertion mutant (referred to as *bx1*) of the inbred line W22 (Tzin et al., 2015) were planted in the field for soil conditioning. To subsequently test benzoxazinoid feedbacks on wheat, we grew the winter wheat variety CH Claro (Agroscope/DSP; referred to as Claro), a variety of top baking quality class (Strebel et al., 2022), and recommended cultivar for cultivation in Switzerland.

2.2 | Field experiment

The field experiment was conducted in Changins (Nyon, Switzerland) on a field at Agroscope (Parcel 29, 46°23″58′ N, 6°14″25′ E; Figure 1) with neutral pH clay loam soil. It consisted of a

maize–wheat rotation, with maize being the conditioning crop and wheat the feedback crop. The field consisted of 10 plots with wild-type maize alternating with 10 plots with *bx1* mutants. Each plot was 6 m long and 3 m wide and separated from each other by 3 m of buffer of alfalfa (*Medicago sativa*). Maize was grown from May to September 2018, followed by wheat from October 2018 to July 2019. The exact timing, field management and previous crops are documented in the Supporting Information: Methods.

2.3 | Sample collection

To investigate soil benzoxazinoid content and to test for effects on the microbes, we collected samples at the end of maize growth and at the beginning and during wheat growth. After maize growth, we sampled root, rhizosphere and soil samples. For that, a soil core (20 × 20 × 20 cm) from one randomly selected plant per plot was excavated and used for chemical and microbial analysis ($n = 10$). At the beginning of the feedback phase, we sampled again soil one day after wheat sowing. At 10 randomly selected positions on each plot, soil cores of the top 20 cm were taken with a 17-mm-diameter soil sampler. Samples of each plot were combined and further processed for chemical and microbial analysis ($n = 10$). We sampled again root, rhizosphere and soil during wheat growth. From 3 randomly selected plants per plot, the root system (7 × 7 cm wide, 12 cm deep) was excavated and pooled for chemical and microbial analysis ($n = 10$). To study within-field variation of soil parameters, soil was sampled after the experiment ended. Soil was taken from five randomly selected positions at a depth of 5–20 cm, resulting in a total of 2 kg of pooled soil per plot ($n = 20$) for chemical analysis.

2.4 | Wheat phenotyping

2.4.1 | Emergence and vegetative growth

One month after sowing, we counted all emerged seedlings along 1 m of three randomly selected wheat rows per plot to determine possible benzoxazinoid soil conditioning effects on wheat emergence. The sum of all counted seedlings per plot was taken, and wheat emergence per area was calculated. To determine the vegetative growth of wheat, we measured plant chlorophyll content, height and biomass accumulation. The chlorophyll content of 15 randomly selected flag leaves per plot was measured with a SPAD-502 chlorophyll metre (Konica Minolta) and the average value was taken for statistical analysis. The height of five randomly selected wheat plants per plot was determined and averaged for statistical analysis. Aboveground biomass accumulation was harvested from 2 × 1 m of wheat row per plot at ground level. Dry weight was determined after the plant material was dried at 80°C until constant weight. The obtained data were used to calculate biomass accumulation per area.

2.4.2 | Insect infestation

Insect infestation was evaluated by counting the number of *Oulema melanompus* larvae during wheat growth. The number of larvae was scored by randomly selecting 5 × 0.5 m of wheat row on each plot and counting larvae present on the flag leaves. In parallel, the number of tillers of these plants was recorded to calculate the number of larvae per plant.

2.4.3 | Insect performance

To further evaluate plant defence, we assessed insect performance on detached wheat leaves. For that, we collected two randomly selected wheat plants per plot and stored them in a zip-lock plastic bag moistened with a wet cotton pad at 4°C, to draw upon as required throughout the insect performance assay. Two transparent solo cups (4 cm height and 3.5 cm diameter) per plot were equipped with a wet filter paper, and the top 6 cm of the youngest fully developed leaf was placed inside. *Spodoptera littoralis* larvae were reared on an artificial diet until used in the bioassay. One healthy third instar larva was preweighed on a microbalance and placed on the wheat leaf before closing the solo cup with an air-permeable lid. Leaves were moistened daily and renewed on days 2 and 4 of the assay to assure excess food for all larvae. Larval performance was evaluated by weighing them after 1 week of feeding. Larvae weight gain per day was calculated (weight end − weight start/number of days feeding × 100), and the mean of the two replicates per plot was used for statistical analysis.

2.4.4 | Harvest

To estimate final plant biomass, we collected again 2 × 1 m of a wheat row on each plot 12 days before harvest. The plant material was dried at 80°C until constant weight and dry biomass were determined. The number of tillers was counted, and plant density and aboveground biomass per tiller were calculated. The wheat was harvested once the kernels were ripe (14% humidity); 9 m^2/plot were harvested with a compact plot combine harvester (Quantum; Wintersteiger), and kernel weight per plot was determined. A subset of these kernels was taken for analysing agronomic kernel quality and food quality-related parameters (described in the Supporting Information: Methods).

2.5 | Soil chemistry

To confirm the gradient of soil chemistry in the field, freshly collected soil was sent to LBU Laboratories (Eric Schweizer AG) and analysed with different extraction methods: water (H_2O), ammonium acetate EDTA (AAE) and carbon dioxide saturated

water (CO_2). H_2O extracts are a proxy for plant-available nutrients, AAE extracts represent nutrients available through plant chelation mechanisms and CO_2 extracts are a common extraction procedure for magnesium, phosphorus and potassium. In addition, total iron was extracted in nitric acid and quantified with inductively coupled plasma mass spectrometry as previously described (Cadot et al., 2021b).

2.6 | Benzoxazinoid analysis

The extraction of benzoxazinoids and their degradation products from soil, as well as the analytical protocol, are detailed in the Supporting Information: Methods, where we also list the measured compounds with their abbreviations, chemical name and where they were sourced from. Benzoxazinoids and degradation products were analysed with an Acquity UHPLC system coupled to a G2-XS QTOF mass spectrometer (Waters AG) as previously described (Gfeller, Waelchli et al., 2023).

2.7 | Benzoxazinoid exudation experiment

To evaluate if maize benzoxazinoid exudation or degradation depends on soil chemical parameters, we performed a climate chamber experiment comparing soils from the north (N: plots WT07/bx04) and south (S: plots WT10/bx07) ends of the field. The soil was sieved (10 mm mesh size) and used to fill 130 mL pots before a wild-type (W22) maize seed was sown ($n = 10$). Plants were grown in walk-in climate chambers under controlled conditions (day/night: 14/10 h; temperature: 22°C/18°C; light 550 µmol/m^2/s; humidity: 60%) and fertilized twice a week with 10 mL of nutrient solution (0.4% [w/v]; Plantactive Typ K, Hauert) supplemented with iron (1‰ [w/v]; Sequestrene rapid, Maag) twice a week. All plants were randomized weekly, watered as needed and harvested after 3 weeks. First, benzoxazinoid exudation was measured by taking a given plant out of the pot, gently removing the soil of the root system at the very bottom from four randomly selected root tips and rinsing 2 cm of the root tips 4× with 100 µL of sterile water. Immediately after, 60 µL of this suspension was added to 140 µL of pure acidified MeOH, resulting in MeOH/H_2O (70:30 [v/v]; 0.1% formic acid). After centrifugation for 10 min at 19,000*g*, the supernatant was stored at −20°C before analysis of benzoxazinoids (as described above). Second, roots were cut at soil level, cleaned off adhering soil with water and dried at 80°C until constant weight. Dry biomass was subsequently determined on a microbalance. Third, the remaining soil in the pot was homogenized, passed through a 5-mm test sieve, 25 mL was put in a 50 mL centrifuge tube and stored at −80°C. Benzoxazinoid extraction and measurement of this soil was done as described above. For statistical analyses, the benzoxazinoid concentrations in the soil were corrected for differences in root dry weight.

2.8 | Benzoxazinoid degradation experiment

To evaluate possible differences in the benzoxazinoid degradation in soils at both ends of the field (N: plots WT07/bx04; S: plots WT10/bx07/WT03), we performed a degradation experiment with labelled deuterated DIMBOA-d_3 under controlled conditions in the laboratory. A 10 mL (≈10 mg) aliquot of this soil was mixed with 10 mL of sterile water in a 50 mL centrifuge tube and blended with a Polytron (30 s at 15,000 rpm), to obtain a homogenous suspension ($n = 4$ per soil). The soil acidity was between pH 6.96 and pH 7.13; therefore, we used a phosphate buffer at pH 7 for the negative and no soil controls. Six millilitres of soil suspension or buffer were transferred into 14 mL culture tubes and incubated at 22°C in a thermoshaker (at 150 rpm) under oxic conditions. We let the soil acclimate for 3 days before the DIMBOA-d_3 was added. DIMBOA-d_3 was dissolved in autoclaved deionized water and added to each culture tube (except negative controls) to obtain a final concentration of 30 µg/mL (≈140 µmol/L). To elucidate the kinetics of benzoxazinoid degradation, we sampled the reaction mixes after 1 min, 7.5 min, 15 min, 1 h, 4 h, 1 day and 4 days. For each sampling point, 300 µL reaction mix was pipetted into a 1.5 mL centrifuge tube containing 700 mL acidified MeOH to result in MeOH/H_2O (70:30 [v/v]; 0.1% formic acid). The suspension was vigorously vortexed and stored at −80°C. Once all samples were collected, the tubes were thawed, soil particles were removed by centrifugation (20 min, 19,000*g*, 4°C), the supernatant was filtered (Target2TM, Regenerated Cellulose Syringe Filters, pore size: 0.45 µm; Thermo Scientific) and stored in a glass vial at −20°C until analysis. Benzoxazinoids were analysed as described above.

2.9 | Microbiota profiling

The Supporting Information: Methods contains the details of sample preparation, DNA extraction and the polymerase chain reaction (PCR) protocol for microbiota profiling. In brief, the collected samples were processed as previously described (Gfeller, Waelchli, et al., 2023), and DNA was extracted using the Spin Kit for Soil (MP Biomedical), following the instructions of the manufacturer. After DNA quantification using the AccuClear Ultra High Sensitivity dsDNA Quantitation Kit (Biotium), bacterial and fungal libraries were constructed largely following our two-step PCR profiling protocol described earlier (Gfeller, Waelchli, et al., 2023). Briefly, bacterial and fungal profiles were based on PCR primer pairs 799-F (Chelius & Triplett, 2001) and 1193-R (Bodenhausen et al., 2013) and ITS1-F (Gardes & Bruns, 1993) and ITS2-R (White et al., 1990), respectively. PCR products were equimolarly pooled followed by ligation of the Illumina adapters by the Next Generation Sequencing Platform at the University of Bern, where they were subsequently sequenced on a MiSeq instrument (v3 cell, paired-end 2 × 300 bp; Illumina). The raw sequencing data are available from the European Nucleotide Archive (http://www.ebi.ac.uk/ena) with the study accession PRJEB59165 (sample IDs ERS14468209 and ERS14468210). The sequencing data

were processed as previously described (Gfeller, Waelchli, et al., 2023). In short, the bioinformatic pipeline includes the following tools: *FastQC* and *cutadapt* (Andrews, 2010; Martin, 2011), *DADA2* (Callahan et al., 2016) and databases *SILVA* v.132 (Callahan, 2018; Quast et al., 2013) and *UNITE* v8.1 (Nilsson et al., 2019) for bacterial and fungal taxonomy. Code and the metadata are available on GitHub (https://github.com/PMI-Basel/Gfeller_et_al_Changins_field_experiment).

2.10 | Statistical analyses

The soil chemical data was analysed and visualized using principal component analysis (PCA; *FactoMineR*; Lê et al., 2008). PC axes were extracted for further analysis. First, the PC axes were used to check for correlations of soil parameters with the field position. Second, in further analysis, the first PC axis, referred to as 'soil chemistry PC1', was factored in the linear models to account for variation explained by soil parameters.

The microbiota analyses are detailed in the Supporting Information: Methods. In brief, microbiotas were analysed using the *vegan* package (Oksanen et al., 2020) for rarefaction analysis (four bacterial and four fungal samples were excluded due to insufficient sequence coverage), unconstrained principal coordinate analysis (PCoA, Bray–Curtis) and permutational analysis of variance (PERMANOVA, 999 permutations; Bray–Curtis). The gradient in soil chemistry was included in all models taking PC1 as a cofactor. Further analyses of alpha- and beta-diversity were performed using the R package *phyloseq* (McMurdie & Holmes, 2013).

Differences in concentrations of soil benzoxazinoids and their degradation products between the two maize genotypes at the end of the conditioning phase, at wheat sowing and during wheat growth were tested by Wilcoxon's rank-sum tests and false discovery rate (FDR) corrected *p* values were reported (Benjamini & Hochberg, 1995), followed by correlation analysis to test for associations between benzoxazinoid concentrations and soil chemistry (using PC1).

Wheat growth and defence-related data were analysed by analysis of variance (ANOVA). Homoscedasticity and normal distribution of error variance were checked visually. For plant phenotypes two different statistical analyses were applied: (i) overall benzoxazinoid conditioning effects, effects of the chemical gradient and the interaction between the two variables were tested with a linear model: lm(phenotype ~ soil conditioning * soil chemistry PC1); (ii) to test for *local* benzoxazinoid-dependent plant–soil feedbacks, we calculated the log-response ratio (LRR) for every plot. This was calculated with the following formulae for wild-type (*log*(*local value*/*surrounding mean*)) and *bx1* (*log*(*surrounding mean*/*local value*)) conditioned plots, where *log*() is the natural logarithm, the *local value* is the realized value of a certain phenotype on the plot of interest, and the *surrounding mean* is the mean of all adjacent plots of the opposite treatment (Supporting Information: Figure S2). The LRRs were then used to test associations between soil parameters and the direction and strength of the feedback, where positive LRRs indicate positive benzoxazinoid plant–soil feedbacks.

In the greenhouse experiment and laboratory degradation experiments, differences between the two soil origins (S, N) were tested by means of Welch's two-sample *t* tests and FDR-corrected *p* values were reported (Benjamini & Hochberg, 1995).

Analyses were conducted using the open-source software R (R Core Team, 2021). Data management and visualization were facilitated with *tidyverse* packages (Wickham et al., 2019). All code for statistical analysis and visualization and the corresponding data can be downloaded from GitHub (https://github.com/PMI-Basel/Gfeller_et_al_Changins_field_experiment).

3 | RESULTS

3.1 | Strong chemical gradient within an experimental field

To assess the soil chemical properties across our experimental field, we measured pH and nutrients in H_2O, CO_2 and AAE extracts on the 20 experimental plots arranged in a grid pattern (Figure 1). PCA revealed a strong chemical gradient. Axis 1, associated mostly with the length of the field, explained 60% of the chemical variation (Figure 2a and Supporting Information: Figure S3), while axis 4 (associated largely with the width of the field) explained 7% of the variation. Overall, we observed the chemical gradient running roughly north–south in a diagonal across the field (Figure 2b). This gradient was also apparent when looking at individual soil nutrients (Supporting Information: Figure S4). It was characterized by elevated levels of Ca (H_2O extracts), K (CO_2 extracts) and Mn, P and Bo (all AAE extracts) towards the northern corner of the field and elevated levels of water-soluble iron and magnesium (all extracts) towards the southern corner. To account for this chemical gradient in the field, we included the PCA axis 1, referred to as soil chemistry PC1, as covariable in all downstream analyses.

3.2 | Benzoxazinoid accumulation covaries with chemical soil gradient

To characterize the conditioning phase (Figure 1), we collected soil samples at maize harvest for benzoxazinoid analysis. We confirmed the presence of benzoxazinoids in soils of wild-type plots while we did not detect them in plots where mutant *bx1* plants were grown (Figure 2c). We measured high amounts of HMBOA and HDMBOA-Glc, followed by MBOA, DIMBOA-Glc and DIMBOA, and low amounts of AMPO in soils of wild-type plots. The benzoxazinoid measurements varied strongly across replicates. Soil levels of several benzoxazinoids, in particular DIMBOA, gradually increased along PC1 (Figure 2c and Supporting Information: Figure S5) from the north to the south of the field. Thus, the innate differences in soil chemistry are associated with marked changes in benzoxazinoid accumulation.

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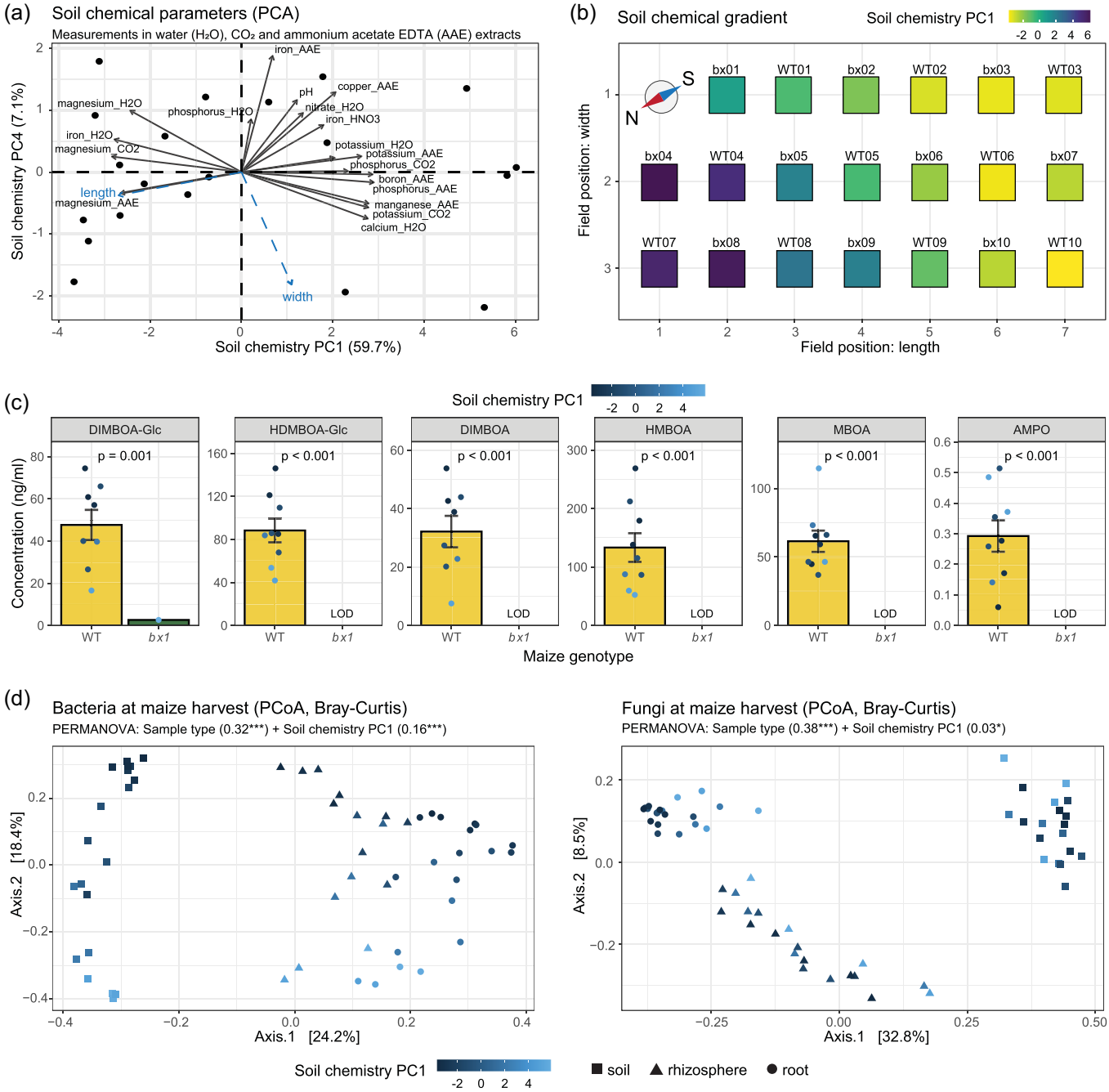

FIGURE 2 Within-field variation of soil chemistry, benzoxazinoids and microbiota. (a) Principal component axes 1 (PC1) and 4 (PC4) of soil chemistry principal component analysis (PCA) are shown. Individual samples (circles), soil parameters (arrows) and direction of field width and length (blue arrows) are included. (b) Field map showing values of soil chemistry PC1 across the field. (c) Soil benzoxazinoid concentrations collected on plots conditioned by wild-type (WT) or benzoxazinoid-deficient *bx1* mutant plants in ng/mL soil (means ± SE). Statistical significance was calculated by Wilcoxon's rank-sum tests and *p* values were corrected for multiple testing. (d) Unconstrained principal coordinate analysis (PCoA) using Bray–Curtis distances of bacterial (left) and fungal (right) communities in soil, rhizosphere and root samples. R^2 and significance level of permutational analysis of variance (PERMANOVA) on Bray–Curtis distances for bacteria and fungi are shown. AAE, ammonium acetate EDTA; CO_2, carbon dioxide saturated water; HNO_3, nitric acid; H_2O, water. Levels of significance: ***$p < 0.001$; *$p < 0.05$.

3.3 | Bacterial and fungal community composition covaries with chemical soil gradients

We also profiled the microbiota of maize roots, its rhizospheres and the soil at maize harvest. Root bacterial communities consisted of abundant Actinobacteria and Alpha- and Gammaproteobacteria, while soils and rhizospheres were characterized by higher amounts of Deltaproteobacteria, Gemmatimonadetes, Chloroflexi, Verrucomicrobia and Acidobacteria (Supporting Information: FigureS6). The maize root fungal community was predominantly composed of

Ascomycota, whereas additional Basidiomycota and Mortierellomycota were abundant in soils and rhizospheres as well as in wheat roots. Again, we noticed that the variation in microbiota composition coincided with the position of the plot in the field along PC1 (Figure 2d). PERMANOVA revealed significant positional effects for the bacteria and the fungi. Taking R^2 values as indicators for effect size, positional effects on bacteria were stronger. The strong positional effects in bacterial communities were apparent in principal coordinate analysis, where the second axis largely separated the replicates following their position along PC1 (Supporting Information: Figure S7). Thus, the innate differences in soil chemistry are associated with differential microbial community composition.

3.4 | Benzoxazinoid exudation shapes root microbiota

To determine whether benzoxazinoids shape microbial communities in maize roots and rhizospheres, as observed before (Cadot et al., 2021b), we first analysed, taking the soil chemical gradient into account, the impact of benzoxazinoids on alpha-diversity. Alpha-diversity of root and rhizosphere bacteria, as well as rhizosphere fungi, were enhanced in wild-type samples relative to *bx1* samples (Supporting Information: Figure S8). We then measured changes in beta-diversity using PERMANOVA to validate benzoxazinoid conditioning and compare the effect size (R^2 values) relative to PC1. Benzoxazinoid exudation shaped microbial communities in the roots and rhizospheres, with stronger effects of soil chemistry than benzoxazinoid effects (Supporting Information: Table S1). Constrained analysis of principal coordinates visually confirmed the effects of benzoxazinoids and soil chemistry on microbial community composition (Figure 3). Thus, benzoxazinoid exudation led to a microbial conditioning.

3.5 | Chemical legacy of benzoxazinoid exudation persists in soil

To test the persistence of benzoxazinoid-dependent effects, we measured benzoxazinoid contents again at wheat sowing and during

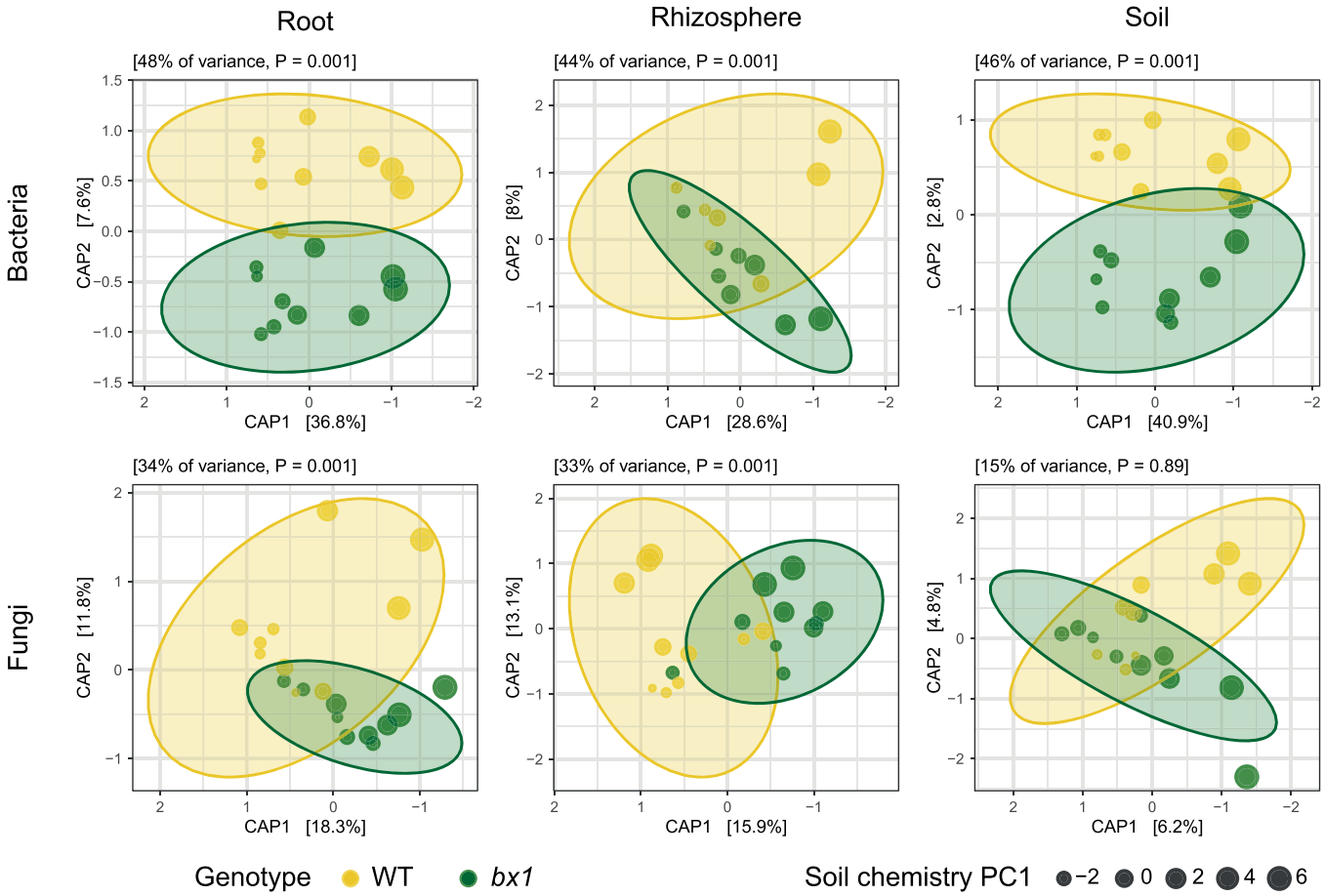

FIGURE 3 Benzoxazinoid-dependent structuring of rhizosphere and root microbiota. Compartment-wise constrained analysis of principal coordinates (CAP) using Bray–Curtis distances of community profiles from bacteria (top) and fungi (bottom). CAPs were performed using the model '~ maize genotype * soil chemistry PC1'. Wild-type (WT) and *bx1* mutant samples are shown for roots, rhizospheres and soils. Data points were sized using the values of soil chemistry principal component axes 1 (PC1). Total variance explained by the model and model significance is shown at the top of each panel. Axis labels indicate the percentage of variance explained.

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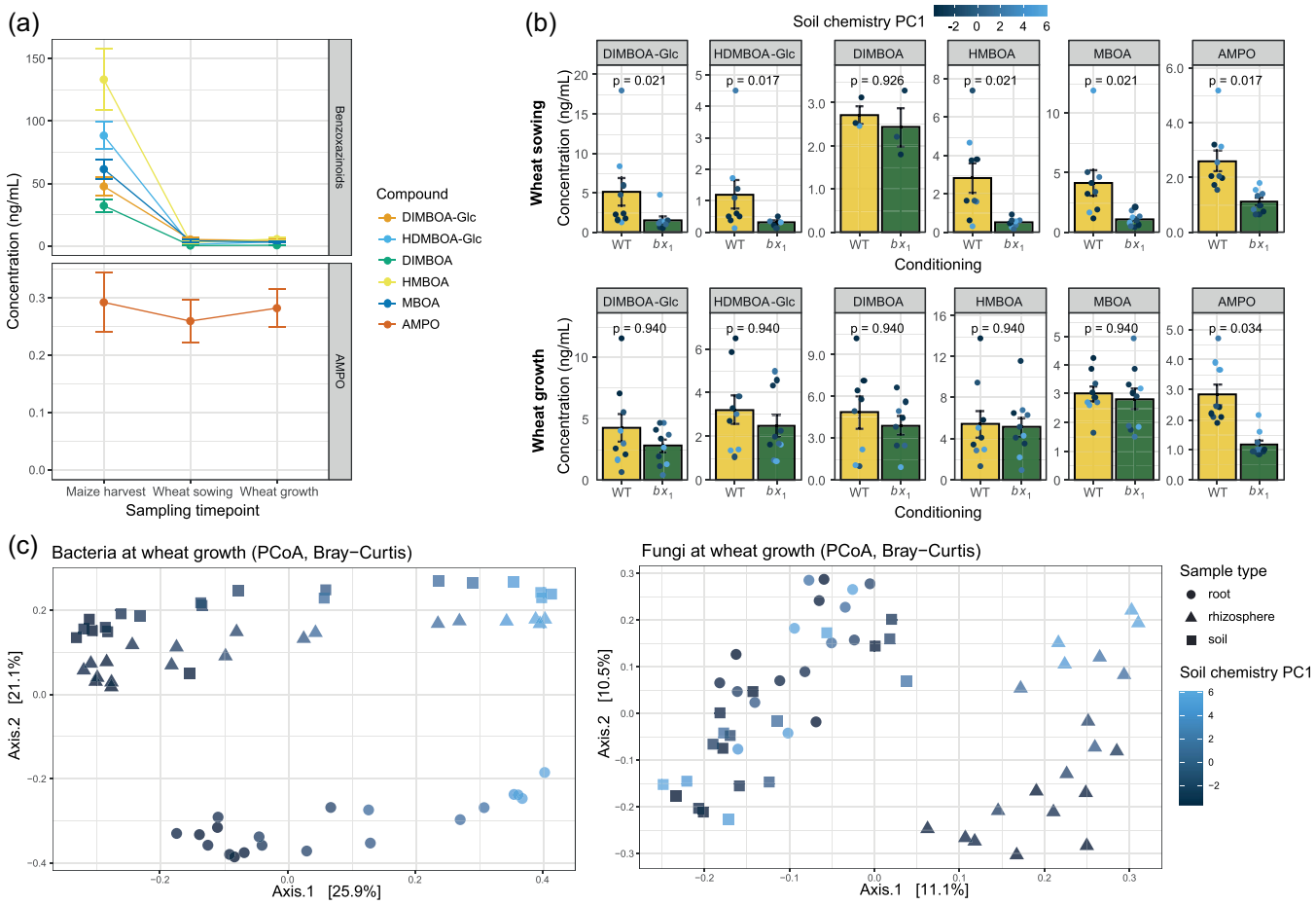

FIGURE 4 Benzoxazinoid-mediated chemical legacy persists in soil. (a) Progression of concentrations of benzoxazinoids and their degradation product (AMPO) in wild-type plots over time (means ± SE). (b) Concentrations of benzoxazinoids in soils collected on plots conditioned by wild-type (WT) or benzoxazinoid-deficient *bx1* mutant plants in ng/mL of soil (means ± SE) at wheat sowing (top) and during wheat growth (bottom). *p* Values were calculated by Wilcoxon's rank-sum tests and corrected for multiple testing. (c) Unconstrained principal coordinate analysis (PCoA) using Bray–Curtis distances of bacterial (left) and fungal (right) communities in root, rhizosphere and soil samples. Axis labels indicate the percentage of variance explained. PC1, principal component axes 1.

To test the persistence of the microbial legacy found at maize harvest (Figure 3), we profiled the soil microbiota at wheat sowing, and the soil, rhizosphere and root microbiotas again during wheat growth. PERMANOVA revealed significant effect sizes for the soil chemical gradient at wheat sowing and during wheat growth (Supporting Information: Table S2). Unconstrained PCoA visualized the structuring of the bacterial communities and of rhizosphere fungi by the soil chemical gradient (Figure 4c). However, no significant impact of benzoxazinoid conditioning on the soil and wheat microbial community composition was detected (Supporting Information: Table S2). Thus, while chemical legacies of benzoxazinoid exudation remained present during wheat growth, microbial legacies disappeared in the feedback phase.

3.6 | Soil chemistry directly determines benzoxazinoid degradation

Differences in soil chemistry may change benzoxazinoid exudation and/or their degradation, thus accounting for the marked gradient in wheat growth in the feedback phase (Figure 1). At wheat sowing, we found 10–100-fold reduced levels of benzoxazinoids compared to our first measurements (Figure 4a). We thus performed analyses on concentrated samples, which also resulted in the detection of low benzoxazinoid levels in *bx1*-conditioned soils. Most benzoxazinoids were still significantly more abundant in soils of wild-type plots (Figure 4b). These quantitative differences were lost during wheat vegetative growth, and some compounds (HDMBOA-Glc, DIMBOA and HMBOA) became more abundant compared to wheat sowing, as wheat also releases benzoxazinoids to the soil (Figure 4b). AMPO, the microbial metabolization product of MBOA, behaved differently than the other benzoxazinoids (Figure 4a): Its concentration decreased only marginally across time points, and it remained significantly higher in wild-type conditioned plots during the entire experiment (Figure 4b). We observed a significant co-variation of AMPO with PC1 (Supporting Information: Figure S9). The concentration gradient of AMPO was always opposite to other benzoxazinoids such as HDMBOA-Glc, DIMBOA and HMBOA (Supporting Information: Figures S5 and S9), suggesting that it may result from differential conversion of benzoxazinoids to their breakdown products.

the directionality of the observed benzoxazinoid accumulation and microbial community composition. To test this hypothesis, we sampled soil from the opposite ends of the soil chemical gradient, that is, south and north soils (Figure 5a). We then grew wild-type maize plants in these soils for 3 weeks and measured benzoxazinoid accumulation in the soil and benzoxazinoid exudation from freshly harvested roots. We did not detect significant differences in benzoxazinoid exudation from roots (Figure 5b). However, we found

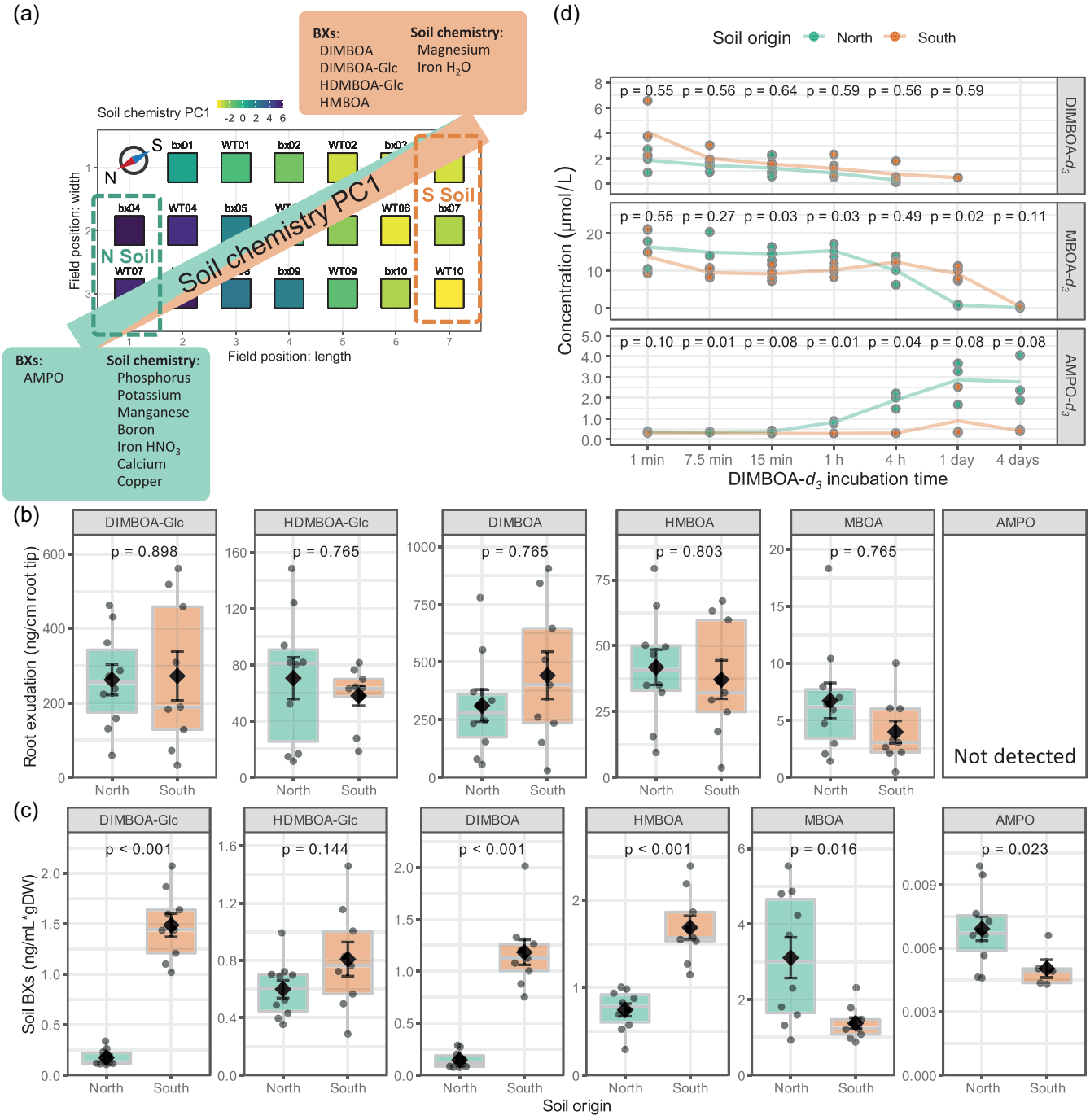

FIGURE 5 Soil chemistry-dependent degradation of benzoxazinoids. (a) Field soils at both extremes of the soil chemistry gradient were collected for benzoxazinoid exudation and degradation experiments under controlled conditions. Enriched chemical parameters in the north and south corners are listed in the mint and orange boxes, respectively. (b) Root benzoxazinoid exudation of 3-week-old maize plants (W22). (c) Benzoxazinoid concentration in soils of 3-week-old maize plants (W22) measured in ng/mL of soil and corrected for root dry weight. (d) Degradation of deuterated DIMBOA-d_3 in a plant-free system monitored for 4 days. For (b) and (c) boxplots, means ± SE and individual data points are shown and for (b)–(d) outputs of Welch's two-sample *t* tests are included (false discovery rate-corrected *p* values). N, north; PC1, principal component axes 1; S, south.

significantly higher benzoxazinoid levels in the south soil compared to north soil (Figure 5c). The glycosylated benzoxazinoids and their conversion products were more abundant in soil of the south compared to the north corner; a finding consistent with the field measurements (Supporting Information: Figures S5 and S9).

To further investigate benzoxazinoid metabolization, we performed an incubation experiment with labelled DIMBOA-d_3 directly spiked in south and north soils and quantified the benzoxazinoid degradation over time. Most of the DIMBOA was rapidly metabolized in the field soils (Supporting Information: Figure S10). In the north soil, DIMBOA was metabolized to MBOA more rapidly, resulting in a faster and stronger accumulation of AMPO compared to the south soil (Figure 5d). In the south soil, almost no AMPO was formed despite the complete metabolization of DIMBOA and MBOA, suggesting that other degradation pathways operate in this corner of the field. Overall, these experiments revealed that benzoxazinoid metabolization is strongly dependent on soil properties, which explains the strong gradient of benzoxazinoids and their degradation products observed across the different plots of the field experiment.

3.7 | Chemical soil gradients are associated with benzoxazinoid-dependent plant–soil feedbacks

To determine the effect of maize benzoxazinoid soil conditioning on the following crop along the soil chemical gradient, we measured wheat performance and resistance in the different plots. For each phenotype, we tested for benzoxazinoid-dependent feedback effects, effects of the soil chemical gradient (PC1), and their interaction. We also quantified the *local feedback* for each plot individually as LRR of wild-type relative to *bx1* soil conditioning at a given location (see Supporting Information: Figure S2). This approach allowed us to compute local benzoxazinoid effects.

Overall, seedling emergence was not affected by soil conditioning or PC1 (Figure 6a). Analysis of local effects, however, revealed a negative effect of benzoxazinoids in plots to the north, and a positive effect in the plots to the south.

During wheat growth, overall chlorophyll content and height were not affected by benzoxazinoid soil conditioning, but local effects were again detected (Figure 6b,c). Positive effects of benzoxazinoids on chlorophyll and height were observed in plots to the north, while negative effects were observed in plots to the south. Plant biomass was negatively affected by benzoxazinoid soil conditioning, with effects that were more pronounced towards the northern end of the gradient in the field (Figure 6d).

As defence-related phenotypes, we counted the number of *Oulema melanopus* larvae on the plants in the field, and we tested the performance of *Spodoptera littoralis* feeding on leaf material collected in the field. Both defence phenotypes were not affected by benzoxazinoid soil conditioning (Figure 6e,f). For *S. littoralis* performance, analysis of local effects revealed a positive effect of benzoxazinoids on larval growth in plots to the north and a negative effect in the plots to the south.

At wheat harvest, no significant benzoxazinoid effects on shoot biomass, biomass per tiller and tiller density were found (Figure 7a–c) and the overall yield was also not affected by benzoxazinoid conditioning (Figure 7d). However, for yield, a weak effect was observed along the gradient, with positive effects in plots to the north and slightly negative effects in plots to the south.

Agronomically important kernel quality parameters, including grain characteristics, protein content and bakeability, were all not affected by overall benzoxazinoid soil conditioning (Supporting Information: Figure S11). Gradients of feedback effects on grain width, volume weight and dough stability were detected. Nutritional and food quality properties were not changed by benzoxazinoid conditioning or along the soil chemical gradient (Supporting Information: Figure S12).

Thus, benzoxazinoid soil conditioning influences wheat growth, defence, yield and grain quality, but the directionality of the effect follows environmental gradients associated with differences in soil chemistry.

4 | DISCUSSION

Soil conditioning by plant secondary metabolites can affect the growth and defence of the following crop through plant–soil feedbacks (Hu, Robert, et al., 2018). If and how such feedbacks depend on the soil type and how soil heterogeneity may influence their spatial patterning remains unknown. Here, we show that the effect of maize benzoxazinoids on wheat performance was fully dependent on soil properties, leading to a distinct effect gradient within a single field. Correlation analysis revealed strong associations between soil parameters (chemistry, bacteria, fungi), soil benzoxazinoid accumulation, and the magnitude and direction of the benzoxazinoid-dependent feedback effects on wheat growth, defence and grain quality. Below, we discuss these findings from a mechanistic perspective and derive implications for the use of secondary metabolite-driven plant–soil feedbacks in agriculture.

4.1 | Impact of soil chemistry on benzoxazinoid accumulation

We find that innate differences in soil chemistry are associated with marked variation in benzoxazinoid accumulation in soil (Figures 2c, 4b and Supporting Information: Figures S5 and S9). Benzoxazinoid exudation can, for example, be altered in response to soil iron (Zhou et al., 2018) and aluminium (Zhao et al., 2019). Soil parameters may also influence the metabolization of secondary metabolites (Nannipieri et al., 2002). The degradation to MBOA, for instance, is pH-dependent (Maresh et al., 2006), and the conversion of MBOA to AMPO, as well as AMPO metabolization, are mediated by soil microbes (Etzerodt et al., 2008; Niemeyer, 2009). Our climate chamber experiments show that the differential accumulation in soils from different field positions is the result of differences in metabolization rather than exudation by maize roots (Figure 5). As benzoxazinoids in the rhizosphere are directly responsible for changes in

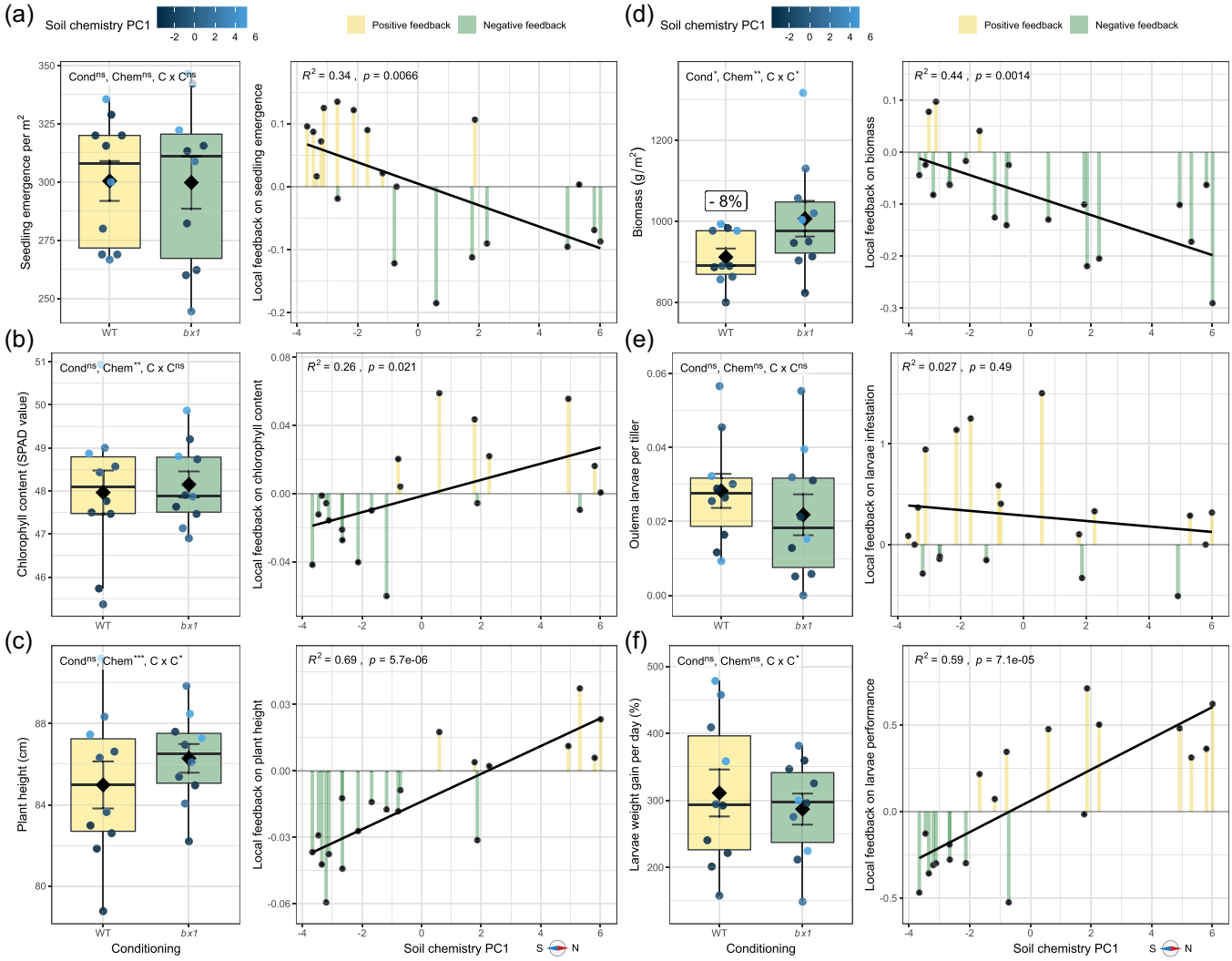

FIGURE 6 Benzoxazinoid-dependent feedbacks during wheat emergence and growth are associated with soil chemistry. (a) Seedling emergence, (b) chlorophyll content, (c) plant height, (d) dry biomass, (e) *Oulema melanopus* infestation and (f) *Spodoptera littoralis* performance during wheat growth. For each phenotype, boxplots (left) and local feedbacks of individual plots along the soil chemistry principal component axes 1 (PC1) (right) are shown. For boxplots, phenotypes measured on plots conditioned by wild-type (WT) or benzoxazinoid-deficient *bx1* mutant maize are shown. Means ± SE and individual data points are included. Further, significance of analysis of variance (ANOVA) output is shown, where benzoxazinoid soil conditioning (Cond), the soil chemistry PC1 (Chem) and their interaction (C × C) were modelled. For the local feedbacks, log-response ratio values of individual plots are shown and R^2 and p value of linear regression are indicated on top. For more details on the local feedback, refer to Section 2. Levels of significance: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{ns}p > 0.05$.

microbial composition and feedback effects on other plants (Hu, Robert, et al., 2018), differences in metabolization may influence plant–soil feedback. The pronounced differences in metabolization we observe within the same field point to the substantial potential for fine-scale variation in secondary metabolite-mediated feedback effects.

4.2 | Interactions between benzoxazinoids and microbiota

Root-associated bacterial and fungal community compositions are well-documented to be affected by benzoxazinoid exudation (Cadot, Guan, et al., 2021; Cotton et al., 2019; Gfeller, Waelchli, et al., 2023; Hu et al., 2018b; Kudjordjie et al., 2019). Here, we confirm this result and show that benzoxazinoid effects on microbiota composition and alpha-diversity are significant, even in heterogeneous soils (Figure 3 and Supporting Information: Table S1). The community structure of fungi, compared to bacteria, was more strongly affected by benzoxazinoids, which is in line with previous findings (Cadot, Guan, et al., 2021). Bacterial communities showed a strong association with soil chemistry, possibly because they respond more dynamically to local changes in environmental conditions. Previous work showed that bacteria are, for example, more strongly affected by soil acidification compared to fungi (Choma et al., 2020; Rousk et al., 2010). Given that benzoxazinoid accumulation is dependent on variation in soil properties and the root and rhizosphere

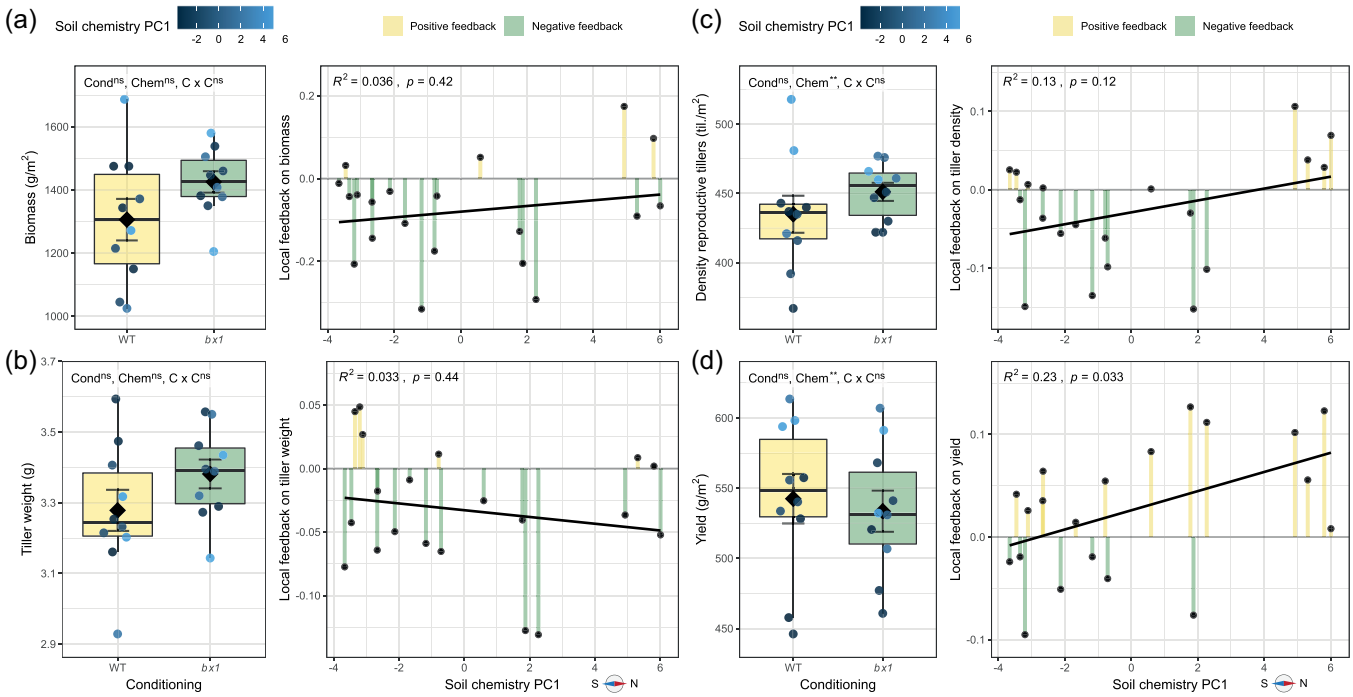

FIGURE 7 Local benzoxazinoid-dependent feedbacks on grain yield at wheat harvest. (a) Biomass, (b) tiller weight, (c) tiller density and (d) yield at wheat harvest. For each phenotype, boxplots (left) and local feedbacks of individual plots along the soil chemistry PC1 (right) are shown. For boxplots, phenotypes measured on plots conditioned by wild-type (WT) or benzoxazinoid-deficient *bx1* mutant maize are shown. Means ± SE and individual data points are included. Further, significance of analysis of variance (ANOVA) output is shown, where benzoxazinoid soil conditioning (Cond), the soil chemistry PC1 (Chem) and their interaction (C × C) were modelled. For the local feedbacks, log-response ratio values of individual plots are shown and R^2 and p value of linear regression are indicated on top. For more details on the local feedback, refer to Section 2. Levels of significance: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{ns}p > 0.05$.

microbiota are shaped by benzoxazinoids, one would expect the benzoxazinoid effect on microbiotas to vary across the field. In our study, we did not observe this behaviour. A possible explanation for this is that root and rhizosphere microbiota are shaped directly by root-exuded benzoxazinoids, which were shown to be unaffected by soil parameters (Figure 5b). In line with our previous field study (Gfeller, Waelchli, et al., 2023), chemical but not microbiota patterns persisted to the next crop generation. This is in contrast to previous pot and container experiments, where microbial fingerprints from the soil conditioning phase were still present during the feedback plant's growth (Hannula et al., 2021; Hu, Robert, et al., 2018). A likely explanation for this discrepancy is that in our experiments, the process of seedbed preparation for wheat with a complete soil homogenization at a depth of 10 cm resulted in the dilution of the microbial fingerprints in the surrounding soil. In summary, both soil chemistry and benzoxazinoid exudation shape root microbiota, which likely adds to the variation and dynamics of plant–soil feedback effects.

4.3 | Within-field variation in plant–soil feedbacks

Plant–soil feedbacks are well known to depend on the environmental context, the responsible mechanisms are, however, only partly understood (Gfeller, Thönen, et al., 2023; van der Putten et al., 2013; Smith-Ramesh & Reynolds, 2017). Plant nutrient supply, for example, can influence the outcome of plant–soil feedbacks in crops and wild plants (Kos et al., 2015a; Kuerban et al., 2022). Generally, it is assumed that increasing soil fertility will weaken the strength of plant–soil feedbacks by lowering soil nutrient feedbacks. This would reduce the plant's dependency on mutualists, and decrease the role of pathogens if plants have more resources to allocate in defence and immunity (Smith-Ramesh & Reynolds, 2017). Here, we found that depending on soil chemistry, the effect of benzoxazinoid-dependent plant–soil feedbacks on growth, defence and food quality differ in strength and/or direction (Figures 6 and 7). During vegetative growth, we found that under more fertile conditions, as indicated by higher wheat yield, benzoxazinoid conditioning led to faster plant growth but less biomass accumulation and lower plant defence. Soil fertility positively correlated with benzoxazinoid degradation and affected microbial community composition. The exact underlying mechanism, however, remains to be investigated. The observed context dependency of plant–soil feedbacks within one field could explain why greenhouse experiments often cannot be reproduced under natural conditions (Forero et al., 2019; Schittko et al., 2016). We further found context dependencies of benzoxazinoid-dependent plant–soil feedbacks between studies: In this field experiment, at the end of wheat vegetative growth, we found an overall negative effect

of benzoxazinoid conditioning on wheat biomass accumulation. This finding is in line with the conclusion of a previous greenhouse experiment (Cadot, Gfeller, et al., 2021) but different from a previous field experiment (Gfeller, Waelchli, et al., 2023). Given that the two field experiments were conducted in different soils at different locations, the observed variation could be explained by benzoxazinoid-dependent plant–soil feedbacks being soil specific, as it was shown in this study and in a maize–maize experiment before (Cadot, Gfeller, et al., 2021). Taken together, our findings show the importance to take into account local variation of plant–soil feedbacks to understand them in diverse environments and further examine their potential in sustainable agriculture.

5 | CONCLUSION

Plants closely interact with their belowground environment. Root-exuded secondary metabolites can directly or indirectly, mediated through changes in the microbiota, affect the next plant generation grown in that soil. Our work shows that such secondary metabolite-mediated plant–soil feedbacks occur within crop rotations under agronomically relevant conditions and that they are highly dependent on the soil chemical context. Consistent with previous work showing that direct effects of benzoxazinoids on aboveground insects depend on soil chemistry (Hu et al., 2021), this study highlights the importance of the local environmental context to drive the effects of plant secondary metabolites. The ultimate and resulting implication for agriculture is the necessity to understand the soil chemical and microbial context dependency of plant–soil feedbacks in crop rotations to make them applicable as a predictable and sustainable practice.

AUTHOR CONTRIBUTIONS

Valentin Gfeller: Conceptualization; data curation; formal analysis; investigation; visualization; methodology; writing—original draft; writing—review and editing. **Selma Cadot:** Conceptualization; data curation; formal analysis; investigation; visualization; writing—review and editing. **Jan Waelchli:** Data curation; formal analysis; visualization; methodology. **Sophie Gulliver:** Investigation. **Céline Terrettaz:** Investigation; writing—review and editing. **Lisa Thönen:** Investigation; writing—review and editing. **Pierre Mateo:** Investigation; writing—review and editing. **Christelle A. M. Robert:** Resources. **Fabio Mascher:** Investigation; resources. **Thomas Steinger:** Investigation; methodology. **Moritz Bigalke:** Investigation; resources; methodology; writing—review and editing. **Matthias Erb**: Conceptualization; formal analysis; resources; methodology; supervision; funding acquisition; writing—original draft; writing—review and editing. **Klaus Schlaeppi:** Conceptualization; data curation; formal analysis; resources; visualization; methodology; supervision; funding acquisition; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw microbiome sequencing data is available from the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under the study accession PRJEB59165 and the sample IDs ERS14468209 and ERS14468210. All other data generated for this study and the R code to reproduce the statistical analyses are deposited on GitHub (https://github.com/PMI-Basel/Gfeller_et_al_Changins_field_experiment).

ETHICS STATEMENT

The authors confirm that they followed the ethical policies of the journal.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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