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Agronomic Performance and Microbial Diversity of Wheat Following Organic and Synthetic Seed Treatments: A Three-Year On-Farm Swiss Field Study

Hassan Mustapha[1] | Josep Massana-Codina[2] | Matthieu Wilhelm[2] | Amandine Fasel[2] | Sonja Reinhard[1] | Robin Sonnard[3] | Raphaël Charles[3] | Charlotte Martin Savoyat[4] | Natacha Bodenhausen[1]

[1]Department of Soil Sciences, Research Institute of Organic Agriculture FiBL, Frick, Aargau, Switzerland | [2]Research Division Plant Protection, Agroscope, Nyon, Vaud, Switzerland | [3]Department Suisse Romande, Research Institute of Organic Agriculture FiBL, Lausanne, Vaud, Switzerland | [4]Proconseil, Lausanne, Vaud, Switzerland

Correspondence: Natacha Bodenhausen (Natacha.Bodenhausen@fibl.org)

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ABSTRACT

Introduction: Environmental stressors on the agricultural field are increasing, from global warming to the rise of the human population. Among the resulting challenges, plant diseases remain one of the most important causes of crop losses worldwide. Chemical pesticides are used to protect crops, yet they often come with significant environmental risks to the farmers, water, pollinators, and soil diversity. It is crucial, therefore, to investigate non-chemical alternatives to protect crops. Nevertheless, their effectiveness often varies and is difficult to predict, as results obtained under laboratory conditions rarely translate to the complex and dynamic environments of agricultural fields.

Materials and Methods: In this 3-year on-farm study, we compared the effects of chemical and alternative seed treatments (Thermoseed, electron beam, and mustard-based treatments) on agronomic parameters (yield, protein, thousand grain weight, and micronutrients) as well as on the bacterial and fungal microbial communities associated with winter wheat.

Results: Neither the chemical nor the alternative seed treatments affected any of the agronomic parameters measured. The treatments had no effect on the microbial community. Because the seeds used had a low disease prevalence, it was not possible to assess the intrinsic efficacy of the alternative treatments.

Conclusions: The alternative treatments showed no evidence of phytotoxicity in our on-farm field study, indicating their suitability for further evaluation. However, none of the treatments, including the synthetic treatment, had a positive effect when seeds carried a low pathogen load. Our results suggest that pesticide applications on seeds with low levels of seedborne pathogens may be unnecessary, unless soilborne pathogens are suspected to be present.

1 | Introduction

Various types of pesticides, including insecticides, herbicides, bactericides, and fungicides, are used in agriculture to protect crops. In 2022, global pesticide use in agriculture reached 3.7 million tons of active ingredients, representing a 4% increase from 2021 and a doubling from 1990 (FAO 2024). Nevertheless, 20% to 40% of global crop yields are still lost due to plant pests and diseases (FAO 2022). With the growing human population, protecting crops and increasing food production remain urgent

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Hassan Mustapha and Josep Massana-Codina contributed equally to this work.

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worldwide issues in the context of the ongoing food crises (FAO, WFP 2009).

On the other hand, reports continue to describe the toxic effects of pesticides on ecosystems (Köhler and Triebskorn 2013). For instance, insecticides used in agriculture affect the behaviour of wild insects and disrupt their pollination patterns (Klatt et al. 2023), while pesticide exposure has been linked to changes in DNA methylation in mayflies (Gouin et al. 2023). Humans are also directly affected by these chemicals, as pesticide residues could be detected on workers handling pesticides used in seed treatments (Han et al. 2021). Such exposure poses significant health risks (Park et al. 2024), particularly since some pesticides are classified as endocrine-disrupting chemicals (Jabłońska-Trypuć et al. 2023). Endocrine disruptors interfere with hormonal signalling and have been correlated with increased risk of tumour development and carcinogenesis (Zeng et al. 2024).

The side effects of pesticides extend beyond animals and humans, reaching the soil, where they can influence the soil microbial communities and their enzymatic activity (Johnsen et al. 2001; Riah et al. 2014). A meta-analysis study has shown that fungicide use not only inhibits the diversity and abundance of soil microorganisms but also significantly decreases their activity (Wang et al. 2025). Residuals of pesticides in soil have been shown to affect the composition of microbial communities, favouring specific taxa over others (Sliti et al. 2024; Maharana et al. 2025). These effects vary depending on the type of pesticide used: organic and synthetic pesticides impact microbial abundance and composition differently (Fournier et al. 2020; Huang et al. 2021). Fournier et al. reported that one biopesticide impacted key taxa in the microbial network, whereas a synthetic pesticide promoted taxa that are less effective at degrading organic compounds. However, in the same study, it was reported that neither pesticide type altered community alpha diversity (Fournier et al. 2020). In contrast, other studies have found that natural and synthetic triketones can affect both alpha and beta diversity of bacterial soil communities when applied at agronomic doses (Romdhane et al. 2019). Moreover, even the same single pesticide (chiral triazole fungicide cis-epoxiconazole) could show a stereoselective negative impact on bacterial and fungal communities and metabolic pathways (Xue et al. 2022). Therefore, this variability limits our understanding of the effect of these pesticides on the soil microbial communities.

Soil biodiversity is positively correlated with nutrient cycling and organic matter decomposition, and it also contributes to plant health by reducing disease incidence (Delgado-Baquerizo et al. 2020). Given its central role in sustaining the terrestrial ecosystem, understanding how pesticides affect the soil microbiome is important (Bardgett and van der Putten 2014). Increasingly, researchers emphasise that the soil microbiome should receive greater attention in ecological studies (Jansson and Hofmockel 2018). The effect of pesticides on the soil microbiome is also impacted by external factors such as time of sampling (Khmelevtsova et al. 2023), the soil chemical and physical properties (Wang et al. 2023), as well as season (Parizadeh et al. 2021; Parizadeh et al. 2023), thus complicating its interpretation.

Chemical fungicide seed treatment against seedborne and soilborne diseases is widely used in many cereal and vegetable crops (Lamichhane et al. 2020). In wheat, seeds are often coated with chemical fungicides to protect them against phytopathogenic fungi, such as those causing seedling blight (caused by *Fusarium* and *Microdochium* species) or bunt diseases (caused by *Tilletia* species), which can reduce both yield and harvest quality. However, while effective against pathogens, chemical seed treatments also have non-target effects on soil microbial communities (Zaller et al. 2016).

Alternatives to chemical seed treatments against cereal seedborne and soilborne diseases include agronomic measures, physical and non-chemical treatments. For example, the use of certified seeds with low levels of pathogen inoculum (Hamelin et al. 2021; Bänziger et al. 2022), or the disinfection of seeds by means of heat treatments (Bänziger et al. 2022) or electron beam irradiation (Li et al. 2025) is effective at limiting the introduction of the pathogen to the field, and thus controls seedborne diseases. It is worth noting that while preventive measures (i.e. seed health tests) and seed disinfection prevent the introduction of seedborne pathogens at planting, these measures do not prevent infection from soilborne pathogens, which can infect the developing seedling during germination. Several non-chemical seed treatments have shown efficacy against wheat seedling pathogens, some of which are effective against both seedborne and soilborne diseases. These include natural substances such as mustard flour (Winter et al. 2001), biological control agents such as *Pseudomonas chlorophalis (*Rose et al. 2003*)* or vaccine-like approaches, such as treating seeds with the modified hypovirulence DT-8 strain of *Sclerotinia sclerotiorum*, which protects wheat crops against *Fusarium* (Tian et al. 2023).

Given the need to protect crops, the impact of chemical treatments, including seed treatments, on the environment and the soil biodiversity, and the complex nature of the effect of these pesticides on the soil biodiversity, we aimed to assess the effect of alternative, non-chemical seed treatments on several agronomic parameters of wheat crops in an on-farm study. In this study, we conducted field trials with winter wheat on 30 farms for 3 years to test the efficacy of alternative seed treatments on disease incidence, and their effect on plant development, yield production and grain quality. We also characterised the impact of seed treatments on rhizosphere bacterial and fungal communities as indicators of soil health.

2 | Materials and Methods

2.1 | Seed and Seed Treatments

Commercial (certified) lots of winter wheat (*Triticum aestivum* L. subsp. aestivum) seed analysed for the presence of pathogenic fungi (*Microdochium* spp. and *Tilletia* spp.) were used in the field trials during the seasons 2021/2022, 2022/2023, and 2023/2024 (Table S1). The variety Montalbano was used in all three seasons, Baretta was used in 2021/2022 and 2022/2023, and Piznair was used in 2023/2024. All varieties are on the Swiss list of recommended varieties (Strebel et al. 2022) and were chosen due to their broad use in the region studied. Seed treatments included an untreated control and four seed treatments: a chemical seed treatment (Coral Extra, Syngenta, containing 25 g/L fludioxonil and 25 g/L difenoconazole) at its recommended dose (2 mL/kg), performed at an industrial site

(ASS, Moudon, Switzerland); a steam treatment regime using Thermoseed technology performed at an industrial site (fenaco Genossenschaft, Lyssach, Switzerland); an accelerated electron beam treatment performed at E-Vita GmbH (E-Vita, Stuttgart, Germany) and a mustard powder treatment (Tillecur, Biofa GmbH, Munsingen, Germany) at its recommended dose (150 g/kg) applied at an industrial site (ASS, Moudon, Switzerland, for conventional producers) or at the farm (in the case of organic producers). The same seed lot (one per year and variety) was used for the different seed treatments and sent to the farmers for sowing in the field trials. Seeds were sown in the fall and harvested at the beginning of summer of the following year. Unless stated otherwise, the year refers to the harvesting year.

2.2 | Field Trials and Assessments

On-farm experiments were conducted from 2021/2022 to 2023/2024. Untreated and treated seeds of the selected variety were planted in single strip trials of at least 9 metres in width and at least 250 metres in length in 30 field sites. Sowing and harvest dates, pre-crop, and other agronomic conditions are summarised in Table S3 for each site. Whenever available, soil type was also added to Table S3. All on-farm experiments were carried out under common agronomic practices, in fields regularly used for cereal production.

Disease assessment for snow mould (*Microdochium* spp.) was performed in the field by assessing plant emergence at the growth stage of BBCH 12-14, a good indicator of treatment efficacy (Bänziger et al. 2022). Four counts of plants were performed in 1-metre-long rows, and averaged. Furthermore, at the end of winter, the percentage of plant coverage and the presence of snow mould disease were assessed visually. The incidence of common and dwarf bunts was assessed visually between the early milky and over-ripe stages (BBCH 73 – 92). The single strips were harvested independently and weighed; a grain sample of each strip was sent to the laboratory for further analysis.

Thousand kernel weight (TKW, g), volume weight ratio (kg/hl) and kernel length, width and area (mm and mm^2) were measured using a Marvin seed analyser (GTA Sensorik, Neubrandenburg, Germany); protein content (%), humidity, hardness, and micronutrient content (calcium, iron, magnesium, phosphor, potassium, zinc) were measured with a near-infrared instrument (NIRFlex N-500, Büchi Labortechnik AG, Switzerland).

2.3 | DNA Extraction and PCR

The microbiome was assessed on a subset of the fields from the experiments performed in 2022-2023 (Table S4). Fields were sampled with a soil auger. The mixed soil samples were sent to Sol-Conseil (https://sol-conseil.ch) for basic soil analysis: soil texture, SOM, pH, nutrients (easily available fraction). Three rhizosphere samples and one soil sample were collected from each field for microbiome analysis. Roots of recently fully emerged plants (5–10 cm plants, BBCH stage 11–12) were cut and placed into sterile 50 mL Falcon tubes. Soil samples were taken between rows, avoiding roots. All samples were stored at −20°C. Roots were washed by adding 35 mL of sterile water to the Falcon tubes for rhizosphere DNA extraction, followed by hand shaking. 25 mL of the mixture was transferred to a new Falcon tube and centrifuged at 4'000 g for 10 min. The pellet was then resuspended with 2 mL of sterile water, and 300 μL of this rhizosphere suspension was transferred into 2 mL tubes containing 1 g of 0.5 mm Zirconia/Silica beads (Biospec products) and kept at −20°C until extraction. For bulk soil DNA extraction, 200 mg (+/−10 mg) of soil was weighed into 2 mL bead tubes of the same type.

DNA was extracted from the soil and rhizosphere with the NucleoMag DNA microbiome kit from Macherey-Nagel (Düren, Germany) following the manufacturer's instructions. A negative control (beads-only sample) was included. The first steps included adding 700 μL of lysis buffer and 150 μL of Enhancer SX, samples were then homogenised with the TissueLyser II (Qiagen) twice for 1 min at 40 Hz. After quantification using the Qubit dsDNA HS Assay Kit (Thermo) in a 384-well plate by reading the fluorescence with the Tecan plate reader (Männendorf, Switzerland), the DNA was normalised to 5 ng/μL concentration.

Amplicon libraries were prepared using two-step PCR with M13 barcodes developed by PacBio. Primers 27F-M13 and 1492R-M13 were used to amplify the 16S rRNA gene and primers ITS1F-M13 and ITS4-M13 were used for the internal transcribed spacer (ITS) region (Table S2). KAPA HiFi HotStart Ready Mix was used for all the reactions with the CFX Opus 384-Real Time PCR System (Bio-Rad Laboratories, Inc.).

For the 16S rRNA gene, the first PCR was done in a total volume of 10 μL and included 300 nM of each primer and 15 ng of extracted DNA. For the ITS region, the first PCR was done in a total volume of 20 μL and included 300 nM of each primer in two replicates of various amounts of DNA (5 and 1.25 ng/reaction). The cycling parameters were as follows: enzyme activation at 95°C for 3 min, two cycles of denaturation at 98°C for 20 s, annealing at 57°C for 16S and 60°C for ITS for 30 s, elongation at 72°C for 90 s, followed by 20 cycles of denaturation at 98°C for 20 s, annealing at 65°C for 30 s, elongation for 90 s at 72°C, and a final elongation at 72° C for 5 min. The PCR product was visualised on a 1% agarose gel.

The second PCR step was to barcode each sample. The total volume was 25 μL and included 300 nm of the barcoded primers, but different volumes of the first PCR (2 μL for 16S and 1 μL for ITS). The cycling parameters were: enzyme activation at 95°C for 3 min, two cycles of denaturation at 98°C for 20 s, primer annealing at 60°C for 20 s, elongation at 72°C for 90 s, followed by 22 cycles of denaturation at 98°C for 20 s, annealing at 65°C for 20 s, elongation for 90 s at 72°C, and a final elongation at 72°C for 5 min. The PCR products were visualised on a 1% agarose gel. The PCR products from the second PCR were purified using a home-made SeraMag magnetic bead solution (Cytiva). After DNA quantification using the NanoQuant Tecan plate reader, samples were pooled in equimolar fashion.

2.4 | Sequencing and Bioinformatics

The sequencing was performed on the next-generation sequencing platform at the University of Bern according to their standard protocol. The fungal community was sequenced on PacBio Sequel II, while the bacterial community was sequenced on PacBio REVIO. The sequencing facility demultiplexed the REVIO sequences. The rest of the analysis was performed on the Scientific Computer Cluster Euler at the ETH Zurich. The Sequel II sequences were demultiplexed with lima 2.7.1 (https://lima.how/).

Amplicons were identified, oriented, and primer-trimmed using the search_pcr command in USEARCH (v11.0.667) (Edgar 2010) with a maximum of two mismatches. This step also removes sequences with multiple amplicons. Trimmed amplicons were then quality-filtered, discarding reads containing ambiguous nucleotides or with a dust-based complexity score exceeding 30%. Reads outside the expected length range were also removed (16S: ≥ 1,350 nt; ITS: 350–900 nt).

First, zero-radius OTUs (ZOTUs) were inferred using the UNOISE3 algorithm (USEARCH v11.0.667; Edgar 2016 a) with a minimum abundance threshold of 9 and simulaltaneous removal of chimeras. Second, ZOTUs were clustered at 97% sequence identity to reduce the influence of intraspecific variation inflated by full-length amplicon sequencing.

Finally, taxonomic assignment was performed with SINTAX (Edgar 2016) and the SILVA SSU v138 database (Quast et al. 2013) and a cutoff of 0.85. The analysis of the SEQUEL II data was similar, except that in silico PCR was performed with primers ITS1ngs (Tedersoo and Anslan 2019) and ITS4 to generate shorter amplicons that cover only ITS1, 5.8S, and ITS2 in order to improve the taxonomic assignment. Taxonomic assignment was performed with SINTAX against the UNITE V10 database (Abarenkov et al. 2010), applying a cutoff of 0.75. Demultiplexed data were deposited at the NCBI Short Read Archive (PRJNA1348435).

2.5 | Statistical Analysis

The *R* statistical environment was used for statistical analysis (*R* Core Team 2025) using RStudio (Posit team 2025). For the agronomic variables (number of plants per metre, yield, protein content and thousand grain weight (TGW)), a linear mixed-effects model was used, with treatment as a fixed effect and year and farm as a random effect. Model assumptions were evaluated using several diagnostic tools, including Q–Q plots to assess normality of the residuals and DHARMa simulation-based diagnostics. Log transformation of the response variable was used to model the average number of plants per metre to improve the fit diagnostics. The code is available in the GitHub repository (https://github.com/fibl/res0sem), while the data is available on Zenodo (https://zenodo.org/records/19335779).

The microbial community data were analysed with the *R* package phyloseq version 4.5 (McMurdie and Holmes 2013) and vegan 2.7–2 (Oksanen et al. 2025). Sequencing depth was assessed through rarefaction plots using the rarecurve function in vegan. Following McMurdie and Holmes (2014), data were not rarefied. For alpha diversity, Shannon diversity was calculated following Jost (2007) using the formula $D = \exp(H)$ where H is estimated with the default index of the diversity function in vegan. The rhizosphere effect was tested with a linear mixed-effect model with farm as a random factor. After sub-setting the rhizosphere samples, the ratio of Shannon for treated versus untreated samples was calculated for each farm. The effect of seed treatments was tested with a linear mixed-effects model with farm as a random effect. For both linear-mixed effect models, homoscedasticity and normal distribution of residuals were confirmed visually with diagnostics plot. The effect of different variables on community composition was tested with the adonis function from vegan, which performs permutational multivariate analysis of variance. Prior to this, homogeneity of dispersion was tested with the betadisper function from vegan. To explore patterns in microbial community composition, PCA (principal component analysis) was performed after Hellinger transformation (Legendre and Gallagher 2001). Redundancy analysis (RDA) was then performed to investigate factors driving the microbial community structure (Borcard et al. 2018).

We use ChatGPT (OpenAI) to assist in polishing the language and improving the clarity of this manuscript.

3 | Results

3.1 | Seedborne and Soilborne Diseases

To compare the effect of seed treatments on agronomic parameters of winter wheat, on-farm experiments were conducted with 30 farmers (Table S3). The fields are located in western Switzerland (Figure S1). They are managed according to a range of Swiss practices, including organic, conventional, or low-input integrated management systems where foliar fungicides are not used. Each farmer conducted one field experiment yearly by sowing a single seed lot without any treatment, or treated with Tillecur (mustard flour), accelerated electron beam (E-beam), and steam (Thermoseed), in single strip plots. Conventional farmers also applied traditional synthetic fungicides, whereas organic farmers, who are not permitted to use synthetic pesticides, could choose an alternative treatment, such as vinegar. We present here the results of 3 years of study. It is worth noting that the field experiments were performed each year on the same farm, but on different fields due to crop rotation.

We first evaluated emergence because snow mould, caused by *Microdochium* sp., results in lower germination, seedling damping-off at emergence, and lower coverage at the end of winter (Cristani 1992; Humphreys et al. 1995). The number of plants per linear metre was counted to evaluate the efficacy of the seed treatments against *Microdochium* seed infection; in addition, the presence of snow mould was assessed visually at the end of winter for snow mould patches. The number of plants per metre varied greatly between farms and years, with the factor year being statistically significant (Figure S2A, Table S5). To highlight treatment effects on germination, plant emergence was expressed relative to the untreated control for each farm and year (Figure 1). Across all treatments, the number of plants varied from slightly higher to slightly lower than their respective untreated control, with most medians close to 0 (i.e., no difference from the untreated control). Interestingly, median values were lower for the mustard-based treatment in 2022 and 2023, although the effect of Tillecur on emergence was not statistically significant (Table S5). There was no interaction between the management system and the treatment's effect.

Regarding disease incidence, snow mould was not observed in any field, and the estimated surface area covered by plants at the end of winter did not differ among the tested treatments (raw data available on Zenodo: https://zenodo.org/records/

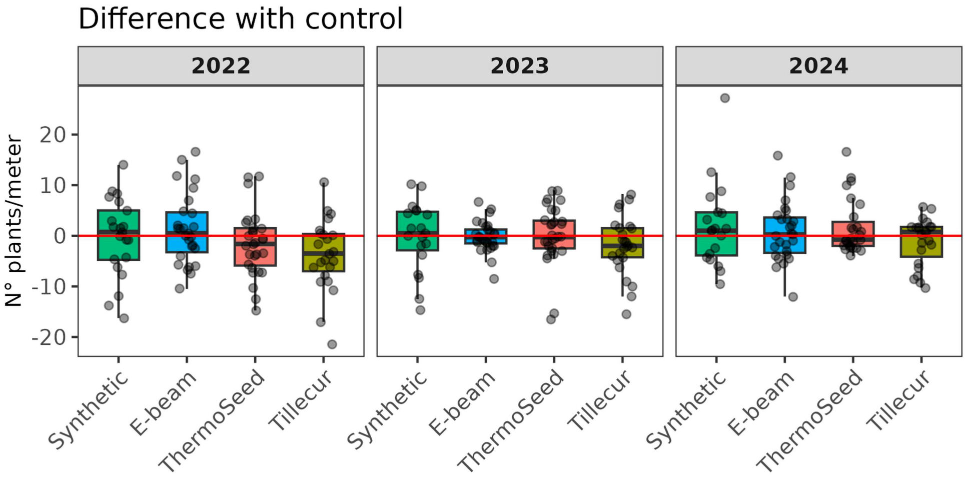

FIGURE 1 | Boxplots of the seed emergence (number of plants per metre) expressed as the difference to the untreated control (UTC) for each treatment across 2022, 2023, and 2024. Boxes represent the interquartile range, and horizontal lines indicate the median. Whiskers extend to 1.5x the interquartile range. Each dot corresponds to an individual observation. Above the red line $y = 0$, there is a positive effect with respect to the control (i.e. the treatment performs better than the control), while below the red line, the effect is negative compared to the control.

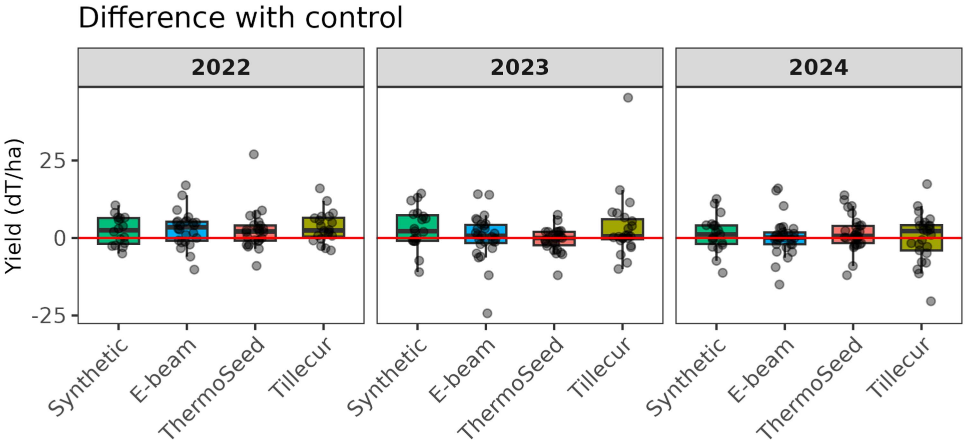

FIGURE 2 | Boxplots of the yield (dt/ha) expressed as the difference to the untreated control (UTC) for different treatments across 2022, 2023, and 2024. Boxplot elements are defined as in Figure 1. Positive values indicate a larger yield than the control. Above the red line $y = 0$, there is a positive effect with respect to the control (i.e. the treatment performs better than the control), while below the red line, the effect is negative compared to the control.

19335779). Furthermore, common and dwarf bunt diseases are only visible during the fruit development and ripening stages (BBCH 73-92). In the field visits during these stages, no bunted ears were observed in any fields, including in the untreated controls. This indicates an overall low prevalence of snow mould and bunt diseases in the assessed fields.

3.2 | Crop Yield and Quality

Next, we examined whether seed treatments influenced yield and quality parameters at harvest. Both agronomic parameters can be affected by seedborne and soilborne diseases, and this evaluation enabled us to determine whether alternative seed treatments impacted harvest performance and yield quality beyond their effect on seedborne diseases. Yield greatly depended on year and farm, ranging from ca. 16 dT/ha to 98 dT/ha (Figure S2B). Lower yields were observed in 2024 (40.5 dt/ha)- a trend observed all over Switzerland due to especially challenging meteorological conditions - while the years 2023 and 2022 yielded 57.3 dt/ha and 63.3 dt/ha, respectively. Farming practices affected the yield, with organic farming showing the lowest average yield (45.8 dt/ha); non-organic, fungicide-free farming yielded 55 dt/ha while conventional agriculture, including foliar fungicide applications, showed the highest average yield with 60 dt/ha.

A comparison with an untreated control in the same farm (Figure 2) showed that treatment effects on yield were minor, ranging from slightly higher to slightly lower than 0, with a tendency towards higher values observed in all seed treatments, especially in 2022. However, the differences in yield in the same farm were relatively minor, and no statistical differences were observed in the dataset (Table S6).

Quality parameters (including protein content, TGW, and micronutrient content) were assessed on the harvested grain across farms, treatments, and years. Seed treatments did not affect the protein content or TGW compared to the untreated controls (Figure 3;

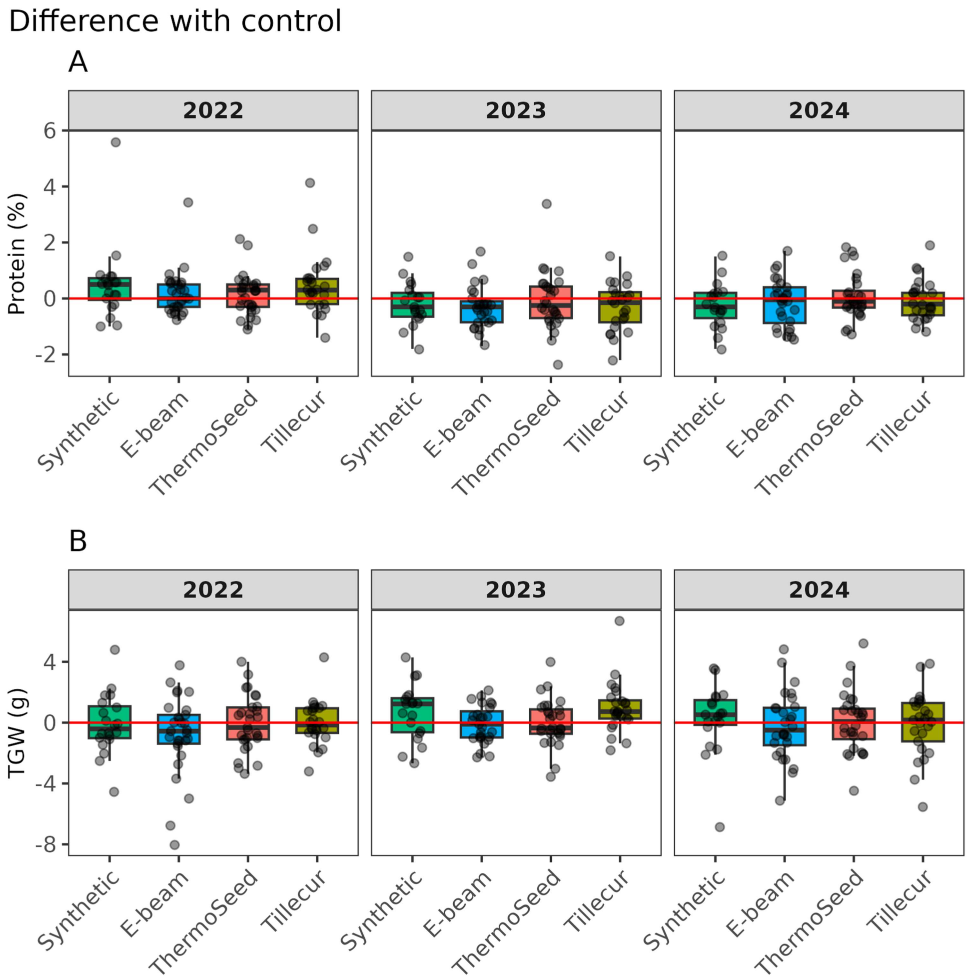

FIGURE 3 | Boxplots representing the quality parameters of the yield from different treatments across the three years, 2022, 2023, and 2024, expressed as a difference to the UTC. (A): protein%, (B): thousand grain weight (TGW). Boxplot elements are defined as in Figure 1. Above the red line $y = 0$, there is a positive effect with respect to the control (i.e. the treatment performs better than the control), while below the red line, the effect is negative compared to the control.

Figure S2C, Figure S2D; Table S7; Table S8). Similarly, all micronutrient content and other parameters measured (Volume/Weight ratio, area, width, and length of seeds, Ca, Fe, Mg, P, K, Zn, humidity, soluble fibre, and insoluble fibre) were comparable between the different treatments and the untreated control (data available on Zenodo: https://zenodo.org/records/19335779).

We asked whether there was an interaction between the production mode and the treatments in the different variables studied. This is of interest since producers interested in using alternative treatments are likely to manage their farms without fungicide, and because the production mode might have an influence on the overall disease pressure. We found no significant interaction between production mode and treatments in any of the three production modes, possibly due to the very low disease pressure in all regions and production modes. The tables summarising the p-values associated with the treatment effect can be found in the supporting material.

3.3 | Alpha and Beta Diversity

In the second year of this study (field season 2022–2023), we investigated the effect of selected seed treatments on the rhizosphere microbial diversity and community structure on a subset of farms. Our hypothesis was that the seed treatments would affect the microbial diversity. Following bioinformatics quality assessment and processing, the number of sequences was higher for the fungal community (2'217'280 sequences, median: 26'308, range: 2'623-69'264), compared to the bacterial community (1'871'911 sequences, median: 2'1897, range: 9'099-40'548). On the other hand, the total number of OTUs was higher for bacteria (5080) compared to the fungal community (2092). Rarefaction plots illustrate the sequencing depth of both bacterial and fungal libraries (Figure S3). The most abundant bacterial phyla among the different farms were Proteobacteria, Acidobacteria, and Bacteroidota, representing 29.5%, 23% and 13.8% of the sequences, respectively. (Figure S4A). Similar to our previous results with the same primer pair (Bodenhausen et al. 2023), Mortierellomycota, Ascomycota, and Basidiomycota were the three most abundant phyla among the fungal community across different farms, representing 35.1%, 33.4% and 14.4% of the sequences (Figure S4B).

We sampled both the rhizosphere and bulk soil to assess the rhizosphere effect. We chose Shannon diversity as a measure of alpha diversity (Figure S5). ANOVA was conducted to test for differences in soil and rhizosphere samples. For the bacterial communities, we observed a rhizosphere effect ($F = 4.0118$, $p = 0.00586$). Bacterial richness was lower in the rhizosphere from the synthetic-treated seeds compared to the soil sample (t-value = 3.055, p-value = 0.0125) and for the Thermoseed-treated seeds (t-value = 3.449, p-value = 0.0040) (Figure S5A). Nevertheless, this rhizosphere effect was not apparent for the untreated control seeds. On the other hand, fungal richness was not influenced by either the rhizosphere effect or the applied seed treatments ($F = 0.717$, $p = 0.584$) (Figure S5B). Results were similar with rarefied data (Figure S5CD).

To test for the effect of treatments on the diversity in the rhizosphere, we calculated the ratio of the Shannon index to the untreated control for each field (Figure 4). The seed treatments reduced the Shannon diversity; however, this effect was not statistically significant (F-value = 4.1324, p-value: 0.279). Similarly, the seed treatments did not affect the rhizosphere fungal alpha diversity (F-value = 1.013, p-value: 0.398). Results were comparable using rarefied data (Figure S6).

Next, we used PCA to investigate the effect of the treatments on the bacterial (Figure S7A) and fungal communities (Figure S7B). The first two PCA axes explained more variance in the bacterial community (42%) compared to the fungal community (18.2%), suggesting greater variability among fungal communities. Samples from the same field generally cluster together. The field effect was confirmed by PERMANOVA analysis (Table S9). A similar pattern was observed for fungal communities, which were also significantly influenced by field (Table S9). Because of the experimental design with only one replicate per treatment per field, it was impossible to assess the effect of seed treatments on beta diversity.

Nevertheless, we wanted to understand what was driving the structure of the microbial communities, so we investigated the influence of the soil properties using RDA. The fields are quite variable (Table S4): for example, pH ranges from 6.3 to 8.1, Corg from 2% to 7.2% and sand from 7.3% to 59.5%. RDA showed that the bacterial community was driven by only two variables: soil pH (19.5% of the variance) and clay (4.1% of the variance). Together, these two parameters explain approximately 23.6% of bacterial community variability (Table S10A). By contrast, for the fungal community, RDA revealed four significant factors shaping the community structure: sand (4.1% of the variance explained), clay (4.8%), Corg (3.0%) and pH (4.0%). These four soil properties explain approximately 15.9% of the fungal community variability (Table S10B). Interestingly, variable selection did not identify phosphorus, potassium, and magnesium as shaping microbial community composition (Table S10A,B).

Visualisation of the RDA results with biplots showed different patterns for the two microbial communities (Figure 5). For the bacterial community, the first axis of the RDA separates the fields according to pH, with the high pH field on the right of the plot. The second axis of the RDA is almost parallel to the arrow for clay, which is confirmed by the fact that the field with the highest clay (BF07, 34.9% clay) is on the lower side of the plot. In the fungal community, Fields BT13 and BT07 are most different from the others on the x-axis, and they are characterised by very high silt (76% and 70.9%), so low sand and clay. The field BT17 has the highest percentage of Corg (7.2%), which only impacts the fungal community.

4 | Discussion

4.1 | Alternative Seed Treatments Do Not Affect Germination and Establishment Under Low Disease Incidence

Snow mould, seedling blight and bunt diseases are the most important seedborne and soilborne diseases of wheat, for which chemical fungicides are commonly used as seed treatment (Lamichhane et al. 2020). In this study, we assessed the performance of non-chemical seed treatments on wheat development in several on-farm trials, in order to determine the impact of these alternative seed treatments under different agronomic and climatic conditions. Snow mould and common and dwarf bunt are the most important seedborne diseases of wheat in Switzerland. Snow mould, caused by *Microdochium* sp. (often referred to as pink snow mould), results in lower germination and can also manifest as seedling blight, foot rot and head blight. Common and dwarf bunt, caused by *Tilletia caries* and *Tilletia controversa*, can result in important yield losses, as the

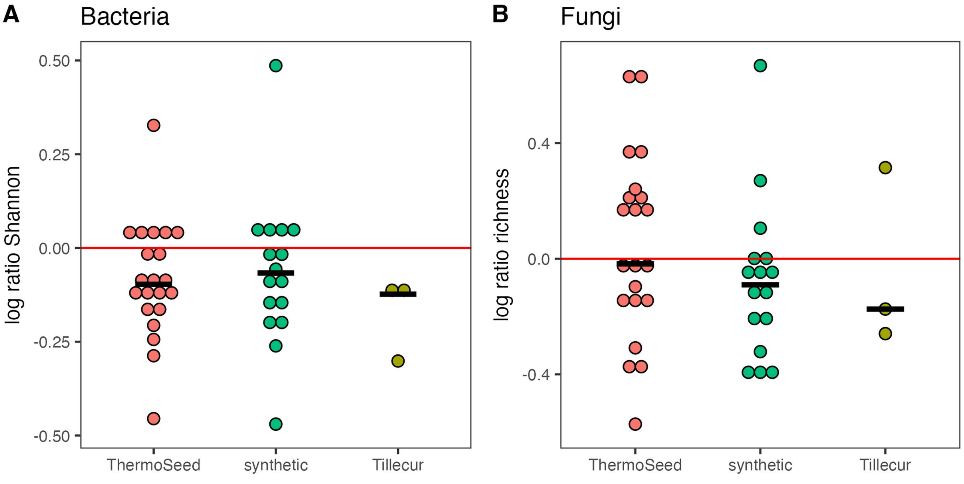

FIGURE 4 | Shannon index plotted as the log ratio of each sample to its respective untreated control, representing the changes in alpha diversity of the bacterial (A) and fungal (B) communities in the rhizosphere. Each dot corresponds to an individual observation. Horizontal black lines represent the median of each distribution.

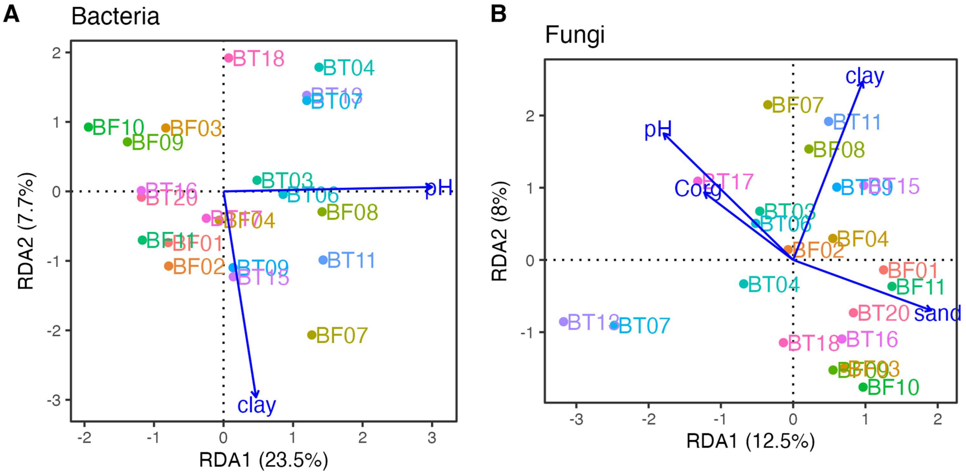

FIGURE 5 | Redundancy analysis (RDA) biplots of microbial communities and soil chemical data for (A) bacterial and (B) fungal communities. Untreated controls of each sampled farm are shown in different colours and indicated by their respective code.

diseased heads are replaced by bunt balls containing the fungal teliospores.

In our study, seedling establishment showed some variability among farms, but seed treatments did not seem to have a clear effect on germination. However, mustard-based treatment showed a slightly reduced number of plants per linear metre compared to other treatments, especially in 2022 (Figure 1), suggesting that Tillecur might slightly negatively affect wheat germination or establishment in some conditions. Indeed, several farmers reported that Tillecur-treated seeds tend to be sticky, which might reduce sowing density and lead to lower emergence ratings in the field. We hypothesise that this explains the observed effect, especially since it was more pronounced in the first year of the project, while in subsequent years, farmers adjusted their practices to ensure more homogeneous density. By contrast, the absence of a strong effect of the other treatments on emergence rate and the lack of differences between control and treatments throughout the season indicate that these alternative treatments do not have a phytotoxic effect. Notably, seeds treated with the chemical fungicide, with a known effect on snow mould, did not result in higher seedling establishment, indicating that *Microdochium* infections were rare in the fields of the network.

Similarly, no snow mould patches at the end of winter nor symptoms of head blight or bunt diseases were observed during plant and ear development. This is consistent with the low infection levels of the seed lots (Table S1). Indeed, the use of clean seeds is a pillar of the Integrated Pest Management (IPM) strategy, and seed health tests are an effective tool for preventing the introduction of diseases in the field (Vishunavat et al. 2023). In Switzerland, the risk of developing snow mould and bunt diseases is low when planting seeds below the pathogen threshold of 10% (for *Microdochium* infection) or 10 spores/grain (for *Tilletia* sp.) (Bänziger et al. 2023). Since we used certified seeds below the pathogen threshold for both snow mould and bunt diseases, our study confirms the value of seed health tests for preventing these seedborne diseases under a range of agronomic and climatic conditions. As different plots were used each year due to crop rotation, the trials were conducted in 90 distinct soils across three seasons; the absence of snow mould and bunt diseases under these diverse conditions suggests that the presence of seedborne *Microdochium* and *Tilletia* is low in the tested fields. However, the low prevalence of snow mould and bunt diseases in these trials limited our ability to confirm or compare the efficacy of the alternative treatments under on-farm conditions.

4.2 | Yield and Quality Are Not Affected by Seed Treatments in the Absence of Disease

Seed treatments can directly improve yield and quality *via* the fungicidal effect against pathogens; in addition, an indirect effect of fungicide beyond disease control has been suggested to increase yield (Turkington et al. 2016). However, the effect of fungicide seed treatments on winter wheat and its effect on yield and quality depends on multiple factors, including disease incidence: in a study with seeds contaminated with *Fusarium* spp at different seed infection levels, it was found that yield was only increased under high seed infection levels, but not at low or intermediate seed infection levels (May et al. 2010). Other studies have reported the positive effect of seed treatments on yield associated with their effect on seedborne diseases (Schaafsma and Tamburic-Ilincic 2005). While few studies have reported direct promoting effects of chemical fungicides on plants (Dal Cortivo et al. 2017), others have suggested that, in the absence of disease, the chemical fungicide treatment might have a negative effect on the plant by impacting the fungal endophyte community, thus reducing its growth-promoting effect (Ayesha et al. 2021).

In our study, we found that neither the alternative nor the synthetic fungicide treatments significantly affected yield, as none of the seed treatments differed statistically from the untreated control (Table S6). Although no significant differences were observed, median yields of all seed treatments were often higher than those of untreated seeds (Figure 2). Furthermore, seed treatments did not affect the quality parameters measured (such as TGW, protein, and micronutrient content). The lack of differences in yield and grain quality under low disease pressure is in accordance with other studies (May et al. 2010). Overall, our results show that for certified seeds with low disease levels, seed treatment has no substantial overall positive or negative impact on the agronomic performance of wheat crops. The absence of positive effects when treating certified seeds, along with the potential for reduced germination rate as observed in this study, has also been reported for the seed treatment of white Lupin (Alkemade et al. 2022). However, snow mould and bunt diseases (particularly dwarf bunt) can also be transmitted through the soil; therefore, under conditions where soilborne pathogens are present, seed treatments can offer a further protection of seedlings from pathogens (Hoffmann and Waldher 1981).

It is worth noting that seed certification processes vary in different regions, and that certified seeds can still be contaminated with seedborne pathogens. Thus, seed health tests, a field currently in development with the use of faster, more efficient molecular methods, are necessary to ensure low pathogen inoculum (Vishunavat et al. 2023). Furthermore, since fungicide seed treatment also targets soilborne diseases, knowledge about pathogen inoculum in the field is also important (Lamichhane et al. 2020). In situations with high soil pathogen inoculum, and for which no agronomic measures are effective, seed treatments with an effect on soilborne pathogens might still be necessary.

4.3 | Seed Treatments Have No Effect on Alpha Diversity

We assessed the impact of alternative seed treatments on soil biodiversity as an indicator of soil health. In the second year of the study, we used the 16S and ITS metabarcoding approach to characterise the diversity and composition of bacterial and fungal communities in the rhizosphere and bulk soil. Diversity of untreated controls was not different from the soil (Figure S5). This contrasts with the commonly observed pattern reported in many studies, including the seminal work of Bulgarelli et al. (2012). Several factors may explain this difference. First, our study was conducted under field conditions instead of in pots, meaning that most of the soil could be considered rhizospheric. Second, sampling was conducted relatively early after emergence and during winter. Both seasonality (Li et al. 2020) and plant developmental stage (Dibner et al. 2021) are known to shape rhizosphere microbial communities, suggesting that this snapshot was taken before the rhizosphere community had fully matured. It is documented that the exudation rate, a major driver of the rhizospheric microbial communities, is lowest at the seedling stage, and increases until the flowering stage (Aulakh et al. 2001). In addition, previous work has reported an increase in exudation rate of different crops as temperatures increase (Leuschner et al. 2022; Santangeli et al. 2026). Given that our sampling took place at an early developmental stage during a cold early winter, lower exudation could be predicted. This could, in turn, lead to decreased selection on the rhizosphere community. Due to resource limitations, microbiome analyses were performed only during the 2022-2023 season. While this allowed us to assess treatment effects, extending the analysis to other years would have been valuable to assess the consistency of the observed patterns under different climatic conditions.

Both synthetic and Thermoseed treatments reduced the species richness of the bacterial community compared to the untreated control; however, this was not significant (Figure 4). Considering the early sampling stage, the absence of the typically higher diversity of the soil compared to well-established rhizosphere communities, and recent findings that seedborne bacteria are major drivers of early rhizosphere assembly (Garrido-Sanz and Keel 2025), we propose that the observed changes in bacterial diversity may reflect the impact of the seed treatments on seedborne endophytic communities rather than on soil-dwelling bacteria. Recent research has shown that seed fungicides can reduce beneficial seedborne bacteria, which typically help suppress seedborne fungi (Kholostiakov et al. 2025). If a similar interaction occurred in our field study, the inhibition of seedborne bacteria by fungicides could allow seedborne fungi to proliferate. However, since we observed comparable fungal richness between treatments and the untreated control, this effect appears limited in our case. To test this hypothesis, a future study could sequence the seedborne bacteria and fungi in both untreated and treated conditions.

4.4 | Soil Properties Shape Microbial Community Composition

Our study investigated how different parameters influence microbial community structure to identify the strongest driving forces. We visualised and tested differences in bacterial and fungal microbial community structure through an unconstrained ordination analysis. The first two PCA axes explained more than double the variability in bacteria compared to the fungi (Figure S7). This pattern suggests that a broader range of environmental factors influences the fungal community structure, each contributing less to the overall variation.

This study was conducted on farms. This kind of study provides ample advantages, including direct access to real-life situations that would be missing in other forms of field trials or even in laboratory settings. With this experimental design, it was impossible to assess the effect of seed treatment on community composition. In contrast, the habitat (soil vs rhizosphere) and the farm location were major drivers of both bacterial and fungal communities. This finding aligns well with other studies showing that seasonality and habitat can be stronger drivers of microbial community composition than the effect of seed treatment. (Parizadeh et al. 2021), and that farm-specific factors may outweigh the effect of the inoculation with plant-growth-promoting rhizobacteria (Kusstatscher et al. 2020).

In our study, we observed that the major drivers of both microbial communities were the chemical properties of the soil (Figure 5). We observe that 24% of the bacterial diversity could be explained by soil pH and clay. On the other hand, 16% of the fungal community variability could be explained by four factors: sand, clay, Corg, and soil pH. This supports the idea that fungal communities may be more robust to the effects of individual environmental factors and are shaped by a broader range of drivers, including climate (Tedersoo et al. 2014). Further, we found that pH had a much larger effect on the bacterial community than on the fungal community, as has been found by others, which attributes this effect to a narrower range of pH for optimal growth of bacteria as compared to fungi (Rousk et al. 2010).

Our findings align with other studies showing that soil properties significantly influence microbial communities' response to fungicides (Wang et al. 2023). This highlights the importance of replicating seed or crop treatment within the same soil type to distinguish treatment effect from soil-driven variation. In our study, the absence of multiple replicates of the same seed treatment within the same farm and soil limited our ability to isolate these effects, preventing a definitive assessment of how seed treatments influenced community composition.

4.5 | Recommendations for Alternative Seed Treatments Incorporation into Agriculture

Many countries are tightening regulations on synthetic pesticides and seeking to reduce their usage. In 2025, the European Commission amended the approval period of several pesticides, including fludioxonil, the most used fungicide in seed treatments. While several NGOs urged the European Commission to ban this active substance, the use of it was extended, but only until September 2026. Finding alternatives to chemical fungicides is therefore essential, and this study's findings point to promising directions.

In our study, we show that treatment of certified seeds with low pathogen load does not affect the quality of harvest and provides only marginal yield improvement. Given these limited agronomic benefits, together with the potential adverse effects on the environment and human health, omitting seed treatments for certified seeds with low levels of seedborne pathogens is a viable option (Bänziger et al. 2023). This is further confirmed by current practice, where untreated seeds account for approximately 25% of use in Switzerland.

However, it is important to point out that the usage of certified seed varies across different countries (Bogdanović et al. 2015), and seed health status is not always assessed during the certification process. Moreover, producing seeds with low levels of disease might be challenging under specific climatic conditions. The percentage of analysed seed lots being rejected due to pathogen loads exceeding defined thresholds varies annually, largely depending on the climatic conditions of the year, particularly for *Microdochium* sp. While rejected lots typically represent less than 10% in most years, this proportion can rise to as much as 50% in unfavourable years (Bänziger et al. 2023).

Therefore, exploring alternative treatments for contaminated seeds remains relevant. In particular, the development of alternatives to conventional synthetic treatments that are suitable for organic farmers is essential. Given the absence of general phytotoxic effects observed in our on-field study, all tested alternatives appear promising for future investigation. However, the efficacy of these alternatives under field conditions with higher disease pressure still needs to be validated. Furthermore, the potential health impacts of these alternative treatments still require further characterisation. Preventive measures during the handling of treated seeds, such as the use of personal protective equipment, should be implemented, including when biological treatments are applied (Niculita-Hirzel et al. 2026).

5 | Conclusions

Previous research has shown that all three alternative treatments tested here (Tillecur, Thermoseed, and electron beam) can reduce seedborne disease contamination under controlled or semi-controlled conditions (Winter et al. 2001; Bänziger et al. 2022; Li et al. 2025). In addition, Tillecur has been reported to have a disease protection effect against *Fusarium graminearum* infection and mycotoxin buildup in wheat grains in growth chambers (Drakopoulos et al. 2019). Here, we aimed to assess the efficacy of these treatments and their impact on plant development in a large-scale on-farm study. The effects of seed treatment on yield were relatively low and not statistically significant under the low disease pressure observed in this study. Likewise, none of the treatments affected crop quality. The findings suggest that in the absence of seedborne disease, seed treatment provides limited agronomic benefits. Nevertheless, given the low disease prevalence in our study, the efficacy of these treatments under higher disease pressure could not be fully assessed. Further research is therefore needed to evaluate the performance of these alternative seed treatments on contaminated seed lots, particularly with respect to agronomic outcomes and impacts on microbial diversity.

Author Contributions

Josep Massana-Codina, Amandine Fasel, Sonja Reinhard, Raphaël Charles, Robin Sonnard, and Natacha Bodenhausen experimented and collected data. Natacha Bodenhausen and Josep Massana-Codina analysed data with input from Matthieu Wilhelm and Hassan Mustapha. Hassan Mustapha and Josep Massana-Codina wrote the first draft of the manuscripts with input from Matthieu Wilhelm and Natacha Bodenhausen. Charlotte Savoyat and Raphaël Charles designed the research. All coauthors contributed to the manuscript's internal revision process. All authors approved the final version of the paper.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The sequencing data are available on NCBI Short Read Archive under the project name PRJNA1348435.

The agronomic dataset is available on Zenodo: https://zenodo.org/records/19335779.

The data analysis code is accessible through the GitHub repository, https://github.com/fibl/res0sem.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Supporting File: sae270168-sup-0001-Supplementary_round3.docx.