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Bread & Beer

Produktion von Weizen und Gerste bei reduziertem

Input im organischen Landbau

TP B

Schlussbericht Phase I

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Die Verantwortung für den Inhalt dieser Veröffentlichung liegt beim Autor.

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I. Kurzdarstellung

Organic farming faces challenges in optimizing grain quality while minimizing reliance on synthetic fertilizers. Wheat and barley, key cereal crops, require efficient nutrient management to improve protein and starch content. Limited availability of nitrogen (N), phosphorus (P), and potassium (K) in organic systems can impacts grain protein concentration, gluten composition, and starch accumulation. Ensuring sufficient nitrogen during grain filling is particularly crucial, as it influences protein accumulation and quality in wheat, while starch content is essential for barley's malting and brewing properties. A promising strategy is the application of plant growth-promoting rhizobacteria (PGPRs), which enhance nutrient uptake and root development. PGPRs support plants through mechanisms such as biological nitrogen fixation, phosphorus solubilization, phytohormone production, and stress resistance. While some studies report higher grain protein content in wheat and improved starch-to-protein ratio in barley, field results regarding the protein and starch composition remains unclear. Another key factor in grain quality is planting density. Reducing competition among plants may improve nitrogen uptake, leading to higher protein levels in wheat and better starch accumulation in barley. Wider row spacing can also reduce nutrient stress, promoting better grain filling and uniformity. However, results of the quality improvement regardless of yield vary. Further long-term research is needed to determine whether these approaches consistently enhance grain quality or if their benefits depend too much on environmental and management factors, limiting their reliability in organic farming.

The Bread & Beer project focuses on optimizing the production of winter wheat (*Triticum aestivum L.*) and spring barley (*Hordeum vulgare* L.) with low input in organic farming at the experimental stations of the University of Giessen and the University of Hohenheim. These two grains were selected due to their distinct quality requirements: for baking wheat, both protein quantity and quality are critical, while for malting barley, a high starch composition and concentration is the primary quality factor.

To understand how quality and yield characteristics are influenced by different factors, the project investigates the effects of root competition, fertilizer regimes, and seed inoculation on the root system and rhizosphere microbiome, which are known to play a key role in nutrient uptake and plant health. One of the approaches tested is the inoculation of seeds with the plant growth-promoting bacterium *Hartmannibacter diazotrophicus*, which has the potential to enhance nutrient

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availability, support root growth, and influence grain composition. The project also evaluates the impact of fertilizer regimes to determine how varying nutrient availability influences yield and grain quality under organic farming conditions.

A major focus of the study is to investigate whether root competition, nutrient limitations, and microbial inoculation under real field conditions have a measurable impact on the final yield and grain composition of wheat and barley. The effects of these treatments are being recorded through yield measurements and quality assessments (AG Zörb), providing valuable insights into sustainable strategies for improving grain production with minimal external inputs in organic agriculture.

For spring barley, the study showed that seed inoculation with *Hartmannibacter diazotrophicus* ($E19^T$) had a significant impact on protein and starch composition. Inoculation increased total protein concentration, particularly hordein levels, which are key for malting quality. Additionally, the presence of $E19^T$ led to a rise in total starch and amylose concentrations, suggesting a potential benefit for brewing applications. However, the effects varied depending on the location, with some treatments showing inconsistent trends. While these findings highlight the potential of microbial inoculation for improving barley quality, the variability across sites indicates that further research is needed to confirm its reliability under different field conditions.

For winter wheat, $E19^T$ inoculation significantly enhanced total protein concentration and gliadin levels, which play a key role in baking properties. However, the seed inoculation also influenced the gliadin-to-glutenin ratio, favouring gliadin accumulation over glutenin, which could affect dough strength and elasticity. Wider row spacing and the absence of fertilizer further reduced glutenin levels, emphasizing the importance of proper nutrient management in maintaining wheat quality. Additionally, $E19^T$ inoculation increased amylose and total starch content, but its effect was most pronounced when combined with sufficient fertilization.

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II. Eingehende Darstellung

II.1 Verwendung der Zuwendung und des erzielten Ergebnisses im Einzelnen, mit Gegenüberstellung der vorgegebenen Ziele

II.1.1 Arbeitspaket „Anlegen von Feldparzellen am Standort und Aussaat“

Due to the COVID pandemic and the delayed start of the project, no field trials with spring barley (to be sown in March 2020) and winter wheat (to be sown in October 2019) could be conducted in the first year. As direct application (seed-coating) of bacteria onto seeds ensures close proximity to germinating roots, it promotes association and enhances root colonization; therefore, this method was chosen over soil broadcast application, which requires higher bacterial quantities and poses greater costs and potential impacts on the soil microbiome.

In the following years, the field trials were conducted as planned. Field experiments were performed at the organic experimental stations Gladbacherhof (GH) (50◦23′N, 8◦15′E), Giessen University, central Germany, and Kleinhohenheim (KH) (48◦44′N, 9◦11′E), Hohenheim University, southwest Germany, during 2020–2021 (season I) and 2021–2022 (season II) and 2022-2023 (season III). For spring barley an additional field (Heidfeldhof) was added just as a working package for university of Hohenheim. At Gladbacherhof, an 8-year crop rotation with 2-year alfalfa grass (*Medicago sativa*), winter rye (*Secale cereale*), potato (*Solanum tuberosum*), winter wheat *(T. aestivum*), field beans (*Vicia faba*), spelt (*T. aestivum ssp. spelta*), and spring barley (*H. vulgare*) has been cultivated. Potato and spelt were planted in the fields before seeding winter wheat and spring barley, respectively. In contrast, a 5-year crop rotation at Kleinhohenheim with clover grass (*Trifolium spp*.), intensive vegetables, summer cereal, vegetables with low N-requirement, and emmer (*T. dicoccum*) was cultivated, with clover grass always planted before winter wheat and spring barley.

The sowing of winter wheat (WW) and spring barley (SB) was carried out by the research farm staff with the support of project partner employees (and HIWI), with the timing always varying slightly due to weather conditions (Table 1). To avoid cross-contamination through the seed drill, the control seeds were sown first, followed by the seeds coated with *H. diazotrophicus* E19. The seed coating with *H. diazotrophicus* E19 was performed shortly beforehand in Giessen, and the seeds were transported to the trial sites either by car or train. Shipping the seeds by mail during the first year of field trials proved impractical. In all years, the majority of seeds successfully

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germinated (Fig. 1). In the third year, the spring barley variety ODILIA had to be replaced with RGT Planet, as no organically certified seeds of ODILIA were available for purchase.

Table 1: Site description, sowing sates, and crop management at Gladbacherhof and Kleinhohenheim. WW: winter wheat; SB: spring barley

Description	Gladbacherhof	Kleinhohenheim
Elevation Above Sea Level	185 m	444 m
Average Annual Precipitation (2020-2023)	661 mm	632 mm
Average Annual Temperature (2020-2023)	11 °C	10.7 °C
Sowing date		
Season I	20.10.2020 (WW)	06.11.2020 (WW)
	09.04.2021 (SB)	21.04.2021 (SB)
Season II	18.10.2021 (WW)	27.10.2021 (WW)
	25.03.2022 (SB)	23.03.2022 (SB)
Season III	31.10.2022 (WW)	27.10.2022 (WW)
	03.05.2023 (SB)	04.05.2023 (SB)
Crop rotation duration	8 years	5 years
Crops used in the crop rotation	2-year alfalfa grass (*Medicago sativa*), winter rye (*Secale cereale*), potato (*Solanum tuberosum*), winter wheat (*T. aestivum*), field beans (Viciafaba), spelt (*T. aestivum ssp. spelta*), and spring barley (*H. vulgare*)	clover grass (*Trifolium spp*.), intensive vegetables, summer cereal, vegetables with low N-requirement, and emmer (*T. dicoccum*)
Crops planted before winter wheat	Potato (*Solanum tuberosum*)	Clover grass (*Trifolium spp., etc*.)
Crops planted before spring barley	Spelt (*Triticum aestivum* ssp. spelta)	Clover grass (*Trifolium spp*., etc.)

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Figure 1: fields overview

The soil properties of the two fields were thoroughly described by Elfad et al. (2010), Chen et al. (2014), Schulz et al. (2014), and Binacchi et al. (2023). Both sites show Haplic Luvisol (IUSS Working Group WRB 2015) as soil type and have been organically managed since 1989 (GH) and 1994 (KH).

The fertilization of winter wheat was carried out at tillering stage (**BBCH stages 20–29)** using biogas slurry from the Gladbacherhof experimental farm which was transferred and applied by members of the Giessen partner group (AG Schnell). The application was performed with watering cans along the seed rows after the biogas slurry had been thoroughly mixed. The nutrient analysis of the biogas slurry was conducted by an external institute commissioned by the Gladbacherhof experimental farm, which also used the slurry for other experiments (Table 2).

Table 2: Composition of biogas slurry

Parameter	Unit	2021	2022	2023	Average (Standard Deviation)
Dry Weight	%	6.87	7.51	8.63	7.67 ± 0.89
Total N	kg ha^{-1}	107.1	100.2	85.5	97.6 ± 11
Ammonium-N	kg ha^{-1}	54	51.6	36	47.2 ± 9.8
P_2O_5	kg ha^{-1}	36.9	34.9	44.4	38.73 ± 5
K_2O	kg ha^{-1}	170.7	154.5	151.8	159 ± 10.2
MgO	kg ha^{-1}	22.8	25.5	26.4	24.9 ± 1.9

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II.1.2 Arbeitspaket „Bodenprobenentnahme und chemische Parameter“

Soil samples were collected before and after sowing or harvesting using a soil corer. The soil properties of both fields are listed in Table 3.

Table 3: Soil characteristics at Gladbacherhof and Kleinhohenheim at 0 – 30 cm depth (average across multiple years and locations)

Parameter	Unit	Gladbacherhof	Kleinhohenheim
pH	$Log[H^+]$	6.5	6.4
Total N	%	0.14	0.12
C/N Ratio	-	9	10.3
P_2O_5	$mg\ kg^{-1}$	60	141
K_2O	$mg\ kg^{-1}$	150	163
Mg	$mg\ kg^{-1}$	120	110
Zn	$mg\ kg^{-1}$	2.2	3.1
Soil Organic Carbon	%	1.2	1.17

II.1.3 Arbeitspaket “Überwachung des Pflanzenwachstums“

During the growth phase, the plant height of the individual trial variants was monitored and documented with photos. In none of the seasons were visually noticeable differences between the trial variants observed. At the end of the season, various plant parameters (straw yield, grain yield, thousand-grain weight) were recorded for all plots, respectively.

II.1.4 Arbeitspaket “Ertrag und Gesamteiweißkonzentration und Bestimmung von Amylose und Stärke“

At harvest, plants were separated into grains and straw. Grain samples were dried, dehulled and milled with a tube mill (MM301, Retsch, Haan, Germany). The DUMAS combustion method was used to determine the total nitrogen (N) content in the individual flour samples with the aid of the macro elemental analyzer Vario MACRO cube CHNS (Elementar Analysensysteme GmbH, Langenselbold, Germany). The N content was then multiplied by a factor of 5.7 in order to determine the total protein concentration. Amylose content was determined using the

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amylopectin-lectin concanavalin A (Con A) complex method, following the protocol of the amylose/amylopectin assay kit (K-AMYL 06/18, Megazyme, Ireland), scaled to a 2 mL reaction system.

II.1.5 Arbeitspaket "Extraktion der Speicherproteine aus Getreide"

Three different protein fractions were extracted according to a method adopted from Osborne (1907, Rekowski et al., 2020). For the extraction of albumin/globulin, 1 mL of extraction buffer (0.067 M $HKNaPO_4$, 0.4 M NaCl, pH 7.6) was added to 100 mg of the whole flour, mixed in an overhead shaker (Multi Bio RS-24, bioSan, Riga, Latvia) for 5 min at 20 °C, and incubated on ice for 10 min with repeated vigorous vortexing. After centrifugation (13800 *g*, 6 °C, 10 min), the supernatant containing albumins and globulins was transferred into new tubes, and the whole extraction step was repeated two more times. The remaining pellet was then extracted with 0.8 mL of 70% (v/v) ethanol for 5 min at 20 °C in an overhead shaker and centrifuged again at 13800 *g* (6 °C, 10 min) to yield the gliadin (WW) hordein (SB) fraction. Again, the extraction was repeated two more times. After a washing step with 1 mL of dH_2O (distilled water) and centrifugation (13.800 *g*, 6 °C, 5 min), the glutenin (WW) and glutelin (SB) fraction was extracted using 0.8 ml of extraction buffer (2 M urea, 1% (w/v) dithiothereitol, 50% (v/v) 2-propanol, 0.05 M Tris pH 7.5) for 5 min in an overhead shaker at 20 °C, followed by incubation at 60°C for 10 min, cooling to room temperature (RT), and centrifugation at 13.800 *g* (6 °C, 10 min). This fraction was also extracted three times. Finally, all samples were frozen at −20 °C for later use. Two separate technical replicates were performed for each sample. The method developed by Bradford (1976) using bovine serum albumin (BSA) as a calibration reference was used to determine the concentration of proteins from the extracted fractions spectrofotometrically (Specord® 50 Plus, Analytik Jena, Jena, Germany). All samples were measured in duplicate.

II.1.6 Arbeitspaket "Qualitative Analyse der Proteinfraktionen mittels SDS-PAGE "

The SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) was performed with a standard Dual Cooled Vertical Unit (Hoefer SE 600, Hoefer, Taufkirchen, Germany) according to Laemmli (Laemmli ,1970) by using 12% (w/v) polyacrylamide separation gels with the size of 16 × 18 cm and a thickness of 1.3 mm, and a running buffer consisting of 0.192 M glycine, 0.025 M Tris and 0.01% SDS. For each gel, a molecular weight marker ranging from 10

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to 150 kD was included (Sigma, Taufkirchen, Germany). Running conditions were 400 V for 90 min followed by 480 V for 120 min and the system was cooled to 18 °C using a cooling unit. After the run, SDS-PAGE gels were fixed with a 40% (v/v) ethanol, 10% (v/v) acetic acid, stained in a heated 0.025% Coomassie R-250 staining solution, washed in dH2O, and destained in 10% (v/v) acetic acid solution until the gel background was completely decolorized. SDS-PAGE gels were scanned by an image scanner (EPSON perfection 700 Photo, Epson, CA, USA; 300 dpi and 16 bits per pixel, as TIF format). The scanned SDS-PAGE gels were evaluated with the Gel Analyzer 2010a program (http://www.gelanalyzer.com/). The gels were analyzed for their color in the "dark on light" mode. For each lane, all existing bands were detected automatically and corrected manually if necessary. For each lane, the sum of the raw volumes (based on pixel intensity) of all bands was set as 100%, and relative intensities of each individual band were calculated and used for comparison between treatments in order to equalize possible gel to gel staining differences. all samples were analysed in duplicates.

II.1.7 Arbeitspaket “Mehl und Backtest“

Water absorption and dough development time (Farinograph-AT, 810152, Barbender Technologie GmbH & Co. KG; Duisburg, Deutschland) of flour was measured prior to baking experiments. The baking test was performed according to Kieffer et al. (1993) with some modifications (Rekowski et al., 2020). To 160 g of flour 1.6 g of dry yeast, 2.4 g NaCl, 1.6 g sugar, 1.6 g fat and 0.016 g ascorbic acid were added. The quantity of water added was based on the water absorption value obtained by the farinograph. Final amounts of flour were corrected in the way that all flours were adjusted to 14%moisture content for reasons of comparability. All ingredients were added to the kneading chamber, mixed (1 min), kneaded for the determined dough development time (3–5 min) and divided into four portions. The resulting dough was proofed (first proof) at 30 ◦C and relative humidity of 80% for 25–27 min depending on the dough development time. The dough was then shaped by hand into a ball shape and placed in rings (D: 7.5 cm, H: 4.5 cm). The relaxed dough was again proofed (final proof) at 30 ◦C and relative humidity of 80% for 55 min plus 15 min scheduled for the forming of the dough. The dough was baked for 25 min at 210 ◦C in a PICCOLO 1–4 STIR oven (Wachtel GmbH, Hilden, Germany). After one-hour cooling at room temperature, specific volume (Vol Scan Profiler 600, Stable Micro Systems Ltd., Surrey, UK), hardness (Texture Profile Analyse, WINOPAL Forschungsbedarf GmbH, Elze, Germany), and

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average pore area (HP ScanJet5590, Böblingen, Germany) were determined. Freshness retention of samples was estimated after 7 days of storage under CO2 atmosphere by calculating the difference between the hardness of the fresh (1 h after baking) and stored (7 days after baking) bread. Elasticity of the samples was calculated from the ratio of springiness (the tendency of a bread to return to its original shape after it has been compressed) of bread after 7 days of storage to fresh bread (1 h after baking), as determined by a Texture Profile Analyzer (WINOPAL Forschungsbedarf GmbH, Elze, Germany). Baking loss was determined by the mass difference before and after baking. For each sample four technical replicates were used.

I.1.8 Arbeitspaket "Ergebnisse zur Sommergerste"

Statistical analysis by applying Linear Mixed Model (LMM) indicated that only the factors "inoculation", "row spacing", "location" and "inoculation-location interaction" had significant effects on the quality parameters of spring barley In general, the outcomes of all quality parameters were consistent in both seasons in response to the two experimental factors "inoculation" and "row spacing" (Fig. 2).

Inoculation with E19 increased the mean concentrations of most quality parameters in both seasons, and this effect was significant for total protein, hordein, total starch and amylose concentrations, as well as percentage of albumin/ globulin- protein Z (Table 4), despite large variations within treatments, between seasons and among fields (Fig. 2a). Regarding the row spacing, there was no clear trend at first glance in the response of quality parameters to this factor (Fig. 2b), nonetheless, LMM analysis demonstrated that glutelin and amylose concentrations were significantly increased by wider rows (Table 4).

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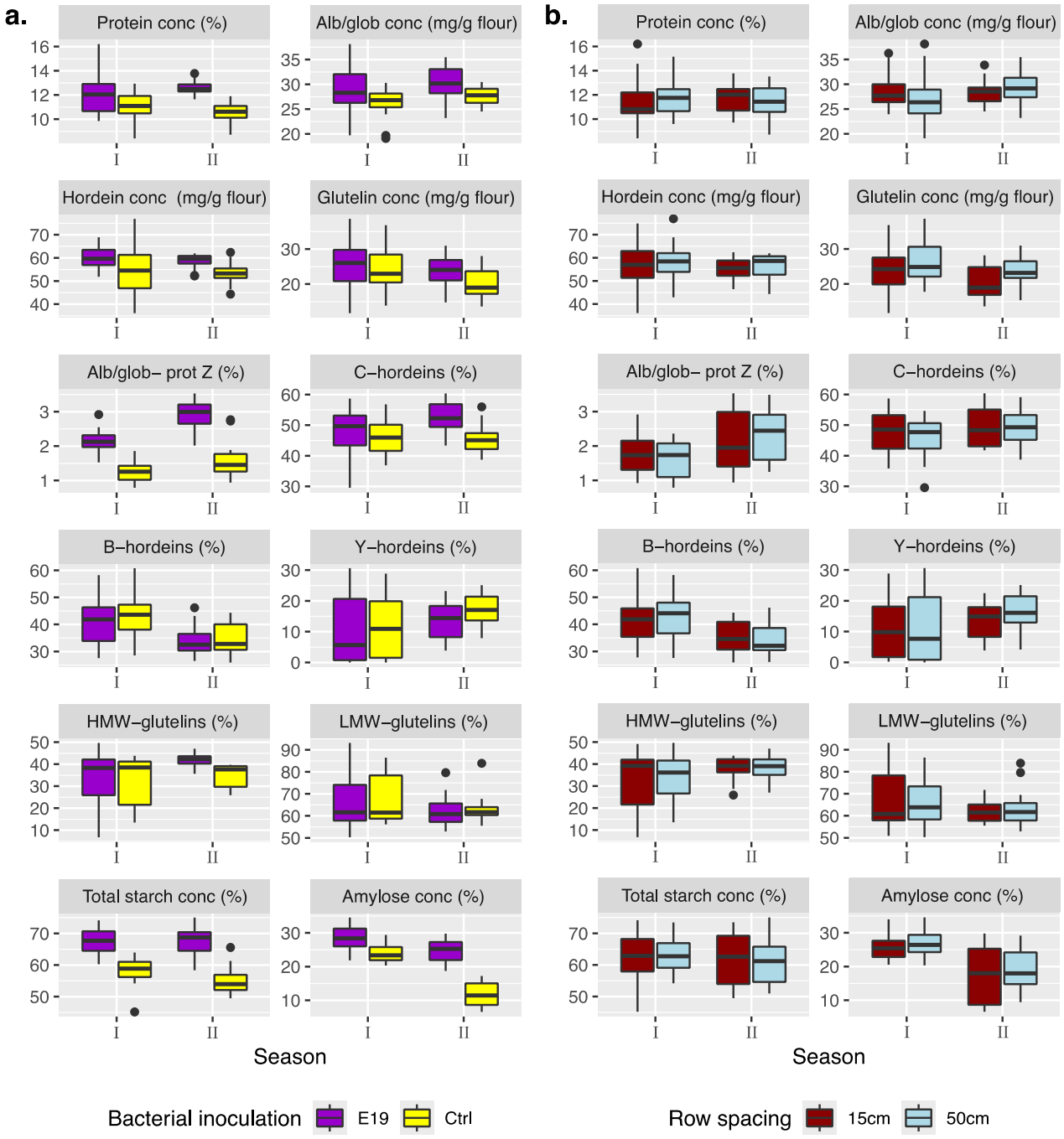

Figure 2: Boxplots of quality parameters of spring barley during the seasons I and II in response to (a) bacterial inoculation (E19: inoculated; Ctrl: non-inoculated) across all three locations and row spacing and (b) row spacing (15 cm and 50 cm) across all locations and inoculated/ non-inoculated.
Albumin/globulin- protein Z (%): Relative share of the Albumin/globulin protein sub-fractions (% of total SDS-PAGE lane intensity). C-hordeins (%), B-hordeins (%), Y-hordeins (%): Relative share of the hordein protein sub-fractions (% of total SDS-PAGE lane intensity). HMW-glutelins

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(%), LMW-glutelins (%): Relative share of the Albumin/globulin protein sub-fractions (% of total SDS-PAGE lane intensity).

Table 4: Result of the type III ANOVA using Satterthwaite's method based on linear mixed models, showing significant effects of different experimental factors on quality parameters of spring barley. Albumin/globulin- protein Z (%): Relative share of the Albumin/globulin protein sub-fractions (% of total SDS-PAGE lane intensity). C-hordeins (%), B-hordeins (%), Y-hordeins (%): Relative share of the hordein protein sub-fractions (% of total SDS-PAGE lane intensity). HMW-glutelins (%), LMW-glutelins (%): Relative share of the Albumin/globulin protein sub-fractions (% of total SDS-PAGE lane intensity).

Parameters		Factor	Bacterial inoculation	Row spacing	Location	Bacterial inoculation* location
Protein concentration	(%)	F value	12.03	--	6.11	5.28
		P	0.009**	--	0.02*	0.03*
Albumin/globulin concentration	(mg/g flour)	F value	6.07	--	8.11	24.24
		P	0.05	--	0.003**	0.001**
Hordein concentration	(mg/g flour)	F value	12.72	--	13.53	3.10
		P	0.004**	--	0.008**	0.08
Glutelin concentration	(mg/g flour)	F value	4.11	6.43	12.30	11.76
		P	0.05	0.01*	0.01*	0.0003***
Albumin/globulin- protein Z	(%)	F value	52.44	--	4.03	--
		P	0.0006***	--	0.05	--
C-hordeins	(%)	F value	4.22	--	0.21	4.25
		P	0.09	--	0.66	0.09
B-hordeins	(%)	F value	1.58	0.0001	15.23	--
		P	0.21	0.99	0.007 **	--
Y-hordeins	(%)	F value	0.65	--	6.86	14.20
		P	0.42	--	0.01 *	0.0003 ***
HMW-glutelins	(%)	F value	4.67	1.53	32.17	0.18
		P	0.07	0.21	0.001 **	0.83
LMW-glutelins	(%)	F value	1.24	0.25	21.75	2.22
		P	0.26	0.61	0.003 **	0.11
Total starch concentration	(%)	F value	150.75	0.009	2.68	1.28
		P	$<2\times10^{-16}$***	0.92	0.16	0.28
Amylose concentration	(%)	F value	16.59	6.92	0.10	--
		P	0.009 **	0.01 *	0.90	--

Empty spaces represent factors that were not included in the linear mixed model. Numbers in red indicate significant effects (p<0.001: "***", <0.01: "**", <0.05: "*"). Interactions of all other factors were not significant.

We further assessed in more detail the effect of bacterial inoculation on all quality parameters by using a "linear mixed model fit by REML", which allowed us to visualize differences of inoculation effects in the three locations (Fig. 3-5).

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Across all locations and row spacings, and compared to the mean of all non-inoculated samples ($\bar{x}$), bacterial inoculation significantly increased the mean total protein concentration by 2.60% ($\bar{x}$ = 10.76; p = 0.001). However, location (p = 0.02) and inoculation x location interaction (p = 0.003; Table 4) also significantly affected total protein concentration, which was increased by inoculation in the two locations GH_{org} and KH_{org}, but not in HH_{conv} (Fig. 3a).

Similarly, inoculation increased hordein concentrations by 9.28 mg/g flour compared to the mean of all non-inoculated samples ($\bar{x}$ = 48.91; p = 0.0007). Additionally, hordein concentrations were significantly affected by location (p = 0.008), but not by inoculation x location interaction (Table 4), in line with an increase in hordein concentrations in all locations by E19, but with a consistently higher hordein level observed in HH_{conv} (Fig. 3c).

Albumins/globulins and glutelins were not significantly affected by bacterial inoculation (p = 0.05; Table 4), but for both parameters the location and inoculation-location interactions were significant. The significant interaction reflects the strong increase observed in GH_{org}, but not in the other locations (Fig. 3b, d). In addition, inoculation even significantly reduced albumin/globulin concentrations by -10.41% in HH ($\bar{x}$ = 25.47, p = 0.001). Glutelin levels were consistently higher in HH_{conv} compared to the two other locations (Fig. 4d) with an average of +11.64% compared to the mean of all samples from the two other locations ($\bar{x}$ = 18.75; $p = 2.06 \times 10^{-5}$).

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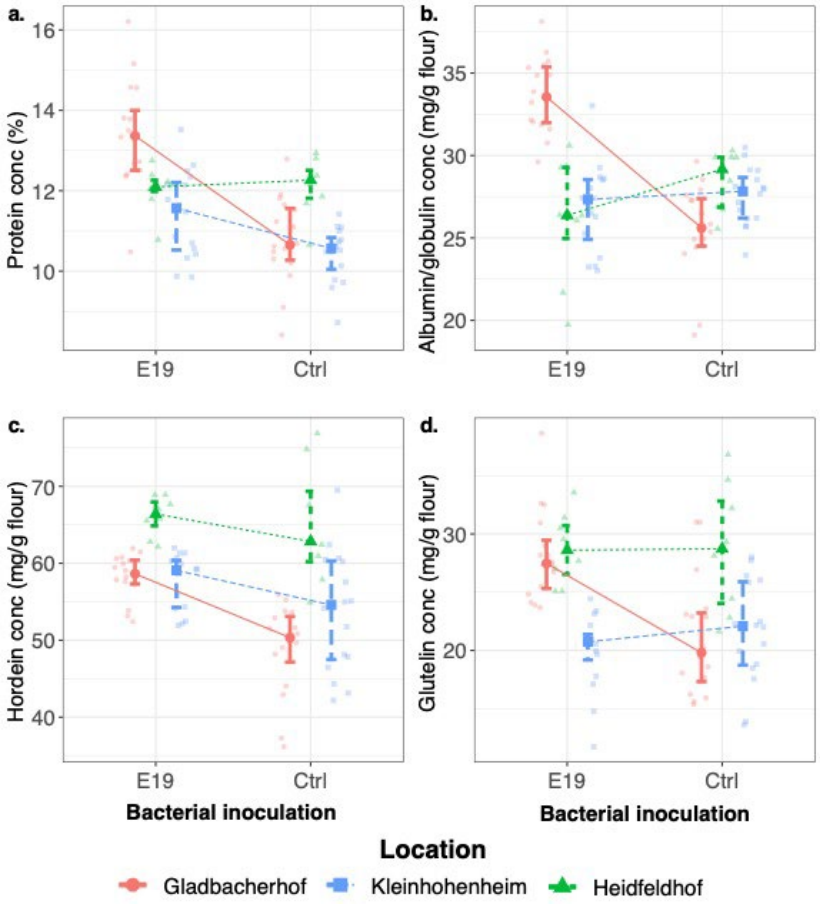

Figure 3. Linear mixed models fit by REML representing the effect of bacterial inoculation on (a) total protein**,** (b) albumin/globulin, (c) hordein, and (d) glutelin concentrations for spring barley in different locations.

Among protein sub-fractions, only albumin/globulin protein Z was significantly affected by E19 inoculation ($p = 0.0006$; Table 4), with an increase in concentration by +1.03% compared to the mean of all non-inoculated samples ($\bar{x} = 1.62$; $p = 0.0006$). The effect of E19 treatment was observed in all three locations, with the steepest increment in KH_{org} (Fig. 4a).

While C-hordeins were not significantly affected by any treatment (Table 4), the effect of location was significant for B-hordeins ($p = 0.007$), HMW-glutelins ($p = 0.001$) and LMW-glutelins ($p = 0.003$), without inoculation x location interactions (all in Table 4). In all cases, levels were consistently higher (B-hordeins and LMW-glutelins) or lower (HMW-glutelins) in HH_{conv} compared to the two other locations (Fig. 4c, e, f) with an average of +15.25% (B-hordeins; $p = 0.002$), +21.86 (LMW-glutelins; $p = 0.0001$) and -18.49 (HMW-glutelins; $p = 0.005$) compared to the means of all samples from the two other locations.

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The effect of the bacterial inoculation on Y-hordeins was significantly affected by location and by inoculation x location interaction (p = 0.0003; Table 4). While Y-hordeins were increased by inoculation at GH_{org}, they were reduced at KH_{org}, and no effect was observed at HH_{conv} (Fig. 4d). the E19 treatment resulted in a significant decrease in Y-hordein levels by -10.95% compared to the mean ($\bar{x}$ = 1.02; p = 0.0003).

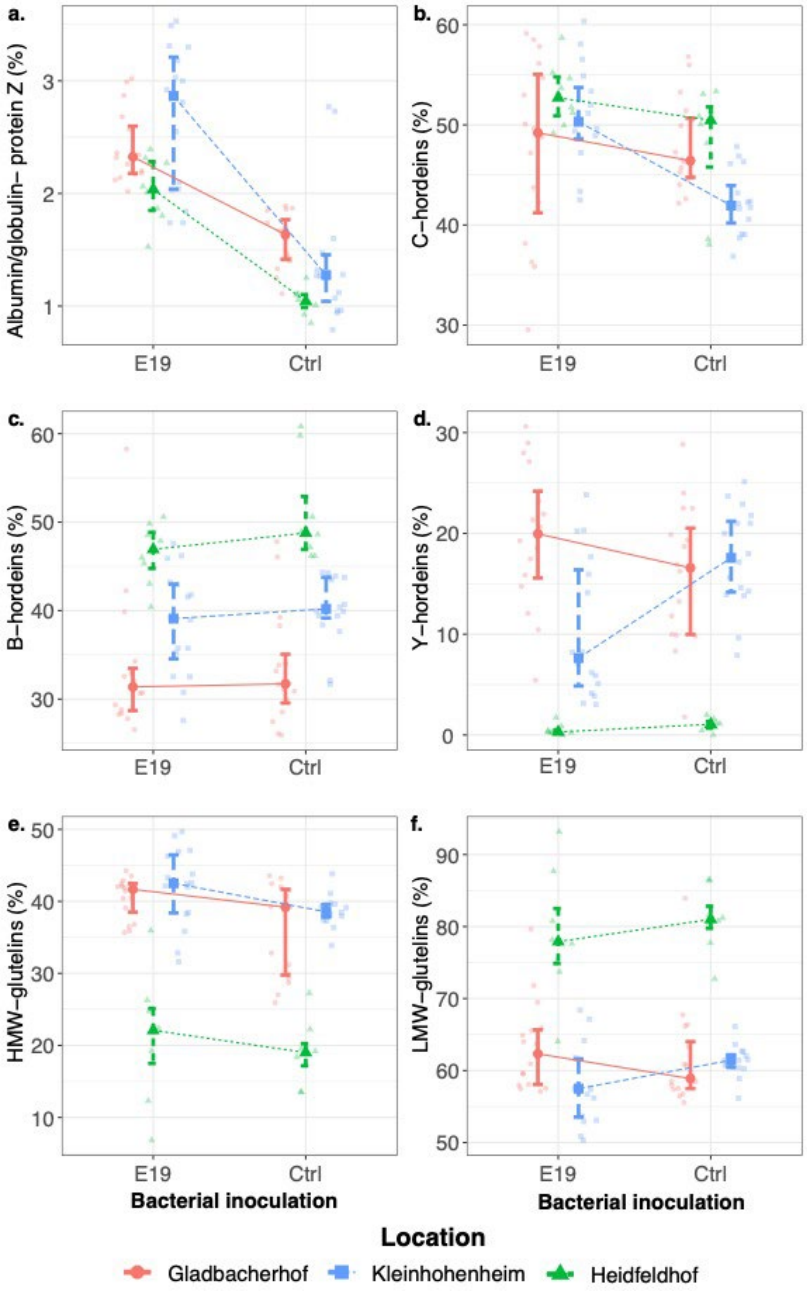

Figure 4. Linear mixed models fit by REML representing the effect of bacterial inoculation on (a) albumin/globulin- protein Z, (b) C-hordeins, (c) B-hordeins, (d) Y-hordeins, (e) HMW-glutelins and (f) LMW-glutelins for spring barley in different locations.

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Bacterial inoculation also significantly affected starch (p = <0.001) and amylose concentrations (p = 0.009; Table 4). Compared to the mean of all non-inoculated samples ($\bar{x}$), bacterial inoculation increased the mean starch concentration by +12.97% ($\bar{x}$ = 57.54; p < 0.001), and the mean amylose concentration by +7.99% ($\bar{x}$ = 18.41; p = 0.009). Both quality parameters were increased similarly by E19 inoculation in all three locations.

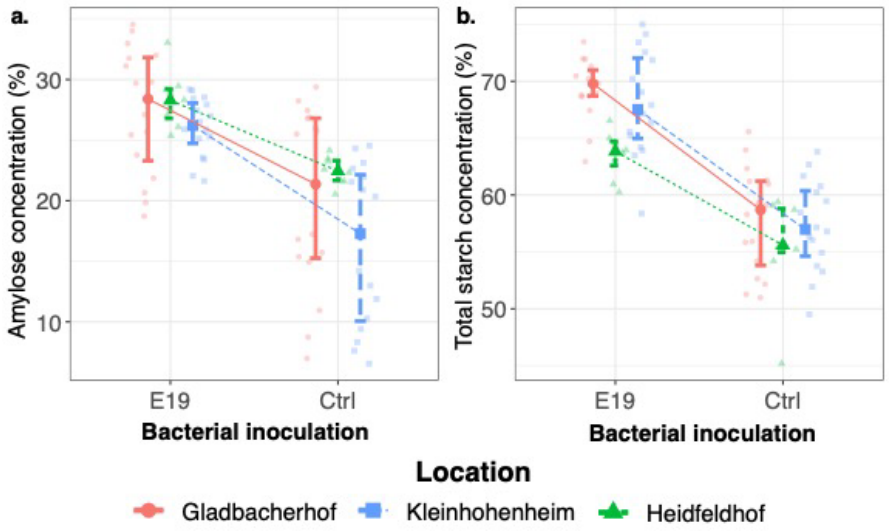

Figure 5. Linear mixed model fit by REML representing the effect of bacterial inoculation on (a) amylose concentration, and (b) total starch concentration for spring barley in different locations.

II.1.9 Arbeitspaket "Ergebnisse zur Winterweizen"

This study evaluated the effects of *H. diazotrophicus* E19^T, fertilizer management, and row spacing on wheat protein and starch quality. The findings indicate that E19^T inoculation significantly increased total protein concentration by 0.55% (p = 0.00575) (Fig. 6a). In contrast, the absence of fertilizer led to a notable reduction in protein content, decreasing it by 1.2% (p < 0.001). The interaction between E19^T and fertilizer suggests that microbial inoculation alone contributes to protein synthesis but is most effective when combined with sufficient nutrient availability.

For gliadin concentration, a significant increase was observed in response to E19^T treatment (p < 0.001), whereas fertilizer application did not lead to a statistically significant change (p = 0.878) (Fig. 6b). This suggests that gliadin synthesis is more influenced by microbial activity than by fertilizer input. additionally, glutenin concentration was negatively affected by wider row spacing (50 cm), resulting in a significant reduction of 8.2 % compared to the mean (p = 0.0016).

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Additionally, the absence of fertilizer led to a reduction of 6.1% compared to the mean ($p = 0.0084$) (Fig. 6c), indicating that adequate fertilization plays a crucial role in maintaining glutenin levels, which are essential for dough strength and elasticity.

The gliadin-to-glutenin ratio was significantly influenced by E19^T inoculation ($p = 0.011$) and fertilizer management ($p < 0.001$) (Fig. 6d). The ratio increased under E19^T treatment and was highest in conditions without fertilizer, indicating a shift in protein composition that could affect baking properties. These results suggest that E19^T favours gliadin accumulation over glutenin synthesis, which may have implications for wheat quality in terms of dough extensibility and loaf volume. Overall, these findings underscore the potential of E19^T inoculation as a tool for enhancing wheat protein content. However, the balance between gliadin and glutenin must be carefully managed to ensure optimal baking quality. While microbial inoculation provides an additional pathway for protein enhancement, it does not fully substitute for fertilizer application.

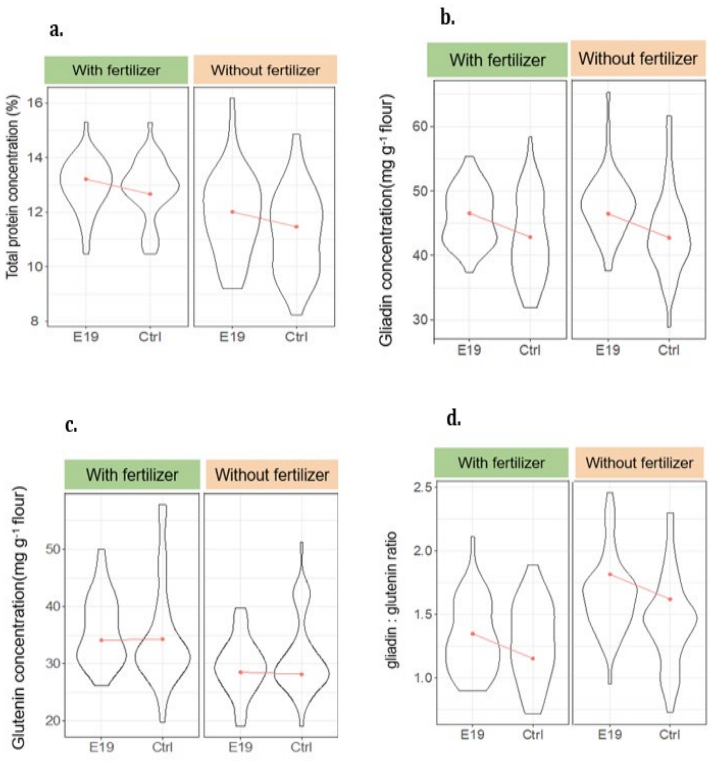

Figure 6. Linear mixed models fit by REML representing the effect of bacterial inoculation on (a) total protein**,** (b) gliadin, (c) glutenin concentrations, and (d) gliadin to glutenin ratio for winter wheat based on different fertilizer management.

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The concentration of ω-gliadin was significantly influenced by $E19^T$, fertilizer management, and row spacing. $E19^T$ inoculation led to a 7.5 % increase compare to the mean ($p < 0.001$) (Fig. 7a). γ-gliadin was significantly affected by row spacing, with wider row spacing leading to lower γ-gliadin levels. However, fertilizer management and $E19^T$ inoculation had no effect on γ-gliadin (Fig. 7b), indicating that this fraction responds more to spatial arrangements than nutrient availability. In contrast, α/β- gliadin showed a strong negative response to $E19^T$ inoculation, with a 7.1 % decrease compare to the mean ($p < 0.001$), suggesting that while $E19^T$ inoculation enhances certain gliadin fractions, it may suppress others (Fig. 7c).

The concentration of HMW-glutenins was significantly increased by $E19^T$ inoculation, with a 4.95 % rise ($p < 0.001$) (Fig. 7d), Fertilizer management also had a positive effect, reinforcing the role of nutrient supply in HMW-glutenin accumulation. However, row spacing alone did not have a significant impact on HMW-glutenins, suggesting that this fraction is more sensitive to biological and chemical inputs than to planting density. D-LMW glutenins were strongly affected by fertilizer application, with a 4.65 % reduction in the absence of fertilizer ($p < 0.001$) (Fig. 7e), indicating that these proteins are highly dependent on nitrogen availability. However, $E19^T$ inoculation had no effect on D-LMW glutenins (Fig. 7e). B-LMW glutenins were significantly affected by both row spacing ($p = 0.0038$) and fertilizer ($p = 0.0046$), with wider spacing leading to lower concentrations and fertilizer application increasing their levels and no $E19^T$ inoculation effect was detected (Fig. 7f). Finally, C-LMW glutenins were significantly influenced by both $E19^T$ ($p = 0.00025$) and fertilizer ($p = 0.0026$) (Fig. 7g), further highlighting the complex interplay between microbial inoculation and nutrient supply in shaping glutenin composition.

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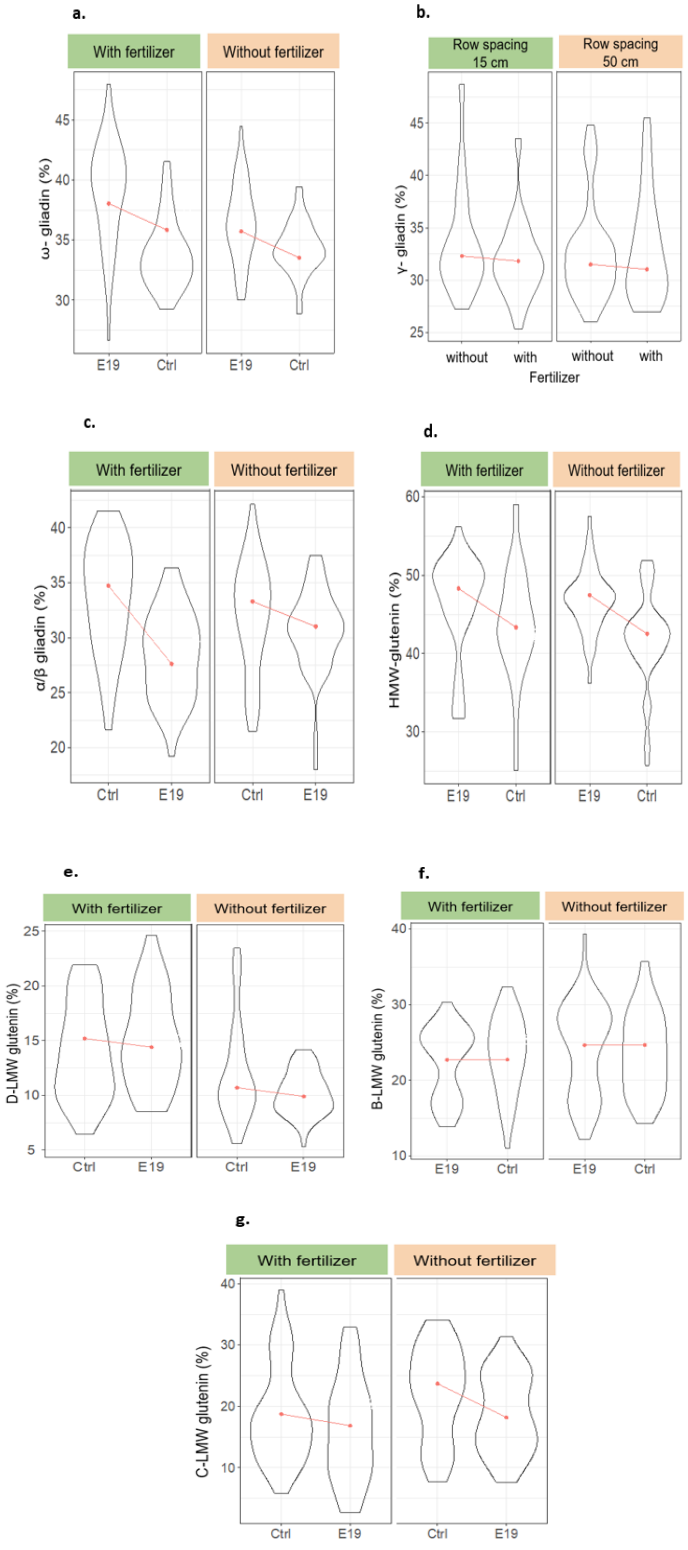

Figure 7. Linear mixed models fit by REML representing the effect of bacterial inoculation on (a) ω-gliadin (b) γ-gliadin, (c) α/β- gliadin, (d) HMW-glutenin, (e) D-LMW glutenin, (f) B-LMW glutenin (g) C-LMW glutenins for winter wheat based on different fertilizer management.

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The analysis of variance (ANOVA) revealed that $E19^T$ inoculation had a highly significant effect on amylose content ($p < 0.001$), increasing its concentration by 2.83% compare to the mean (Fig. 8a). Fertilizer management was also a key factor, with the absence of fertilizer leading to a significant 8.31% reduction in amylose ($p < 0.001$). The interaction between $E19^T$ inoculation and fertilizer management was statistically significant ($p = 0.01149$), indicating that the effects of microbial inoculation on amylose were dependent on nutrient availability. This suggests that while $E19^T$ inoculation E19 can enhance amylose accumulation, its effectiveness is more pronounced under fertilized conditions. $E19^T$ inoculation significantly increased total starch concentration ($p = 0.0024$), with a reported increase of 2.95% (Fig. 8b). Similarly, fertilizer absence resulted in a significant reduction of 2.73% ($p = 0.0096$). The interaction effect between $E19^T$ inoculation and fertilizer management was not statistically significant, implying that both factors independently contributed to starch accumulation. The fixed-effects model indicated that $E19^T$ inoculation significantly increased loaf volume by 9.1% compared to the mean ($p = 0.0256$) (Fig. 8c). However, the absence of fertilizer had a strong negative impact, leading to a 26.9 % reduction ($p < 0.001$), demonstrating the importance of nitrogen availability for achieving optimal bread volume.

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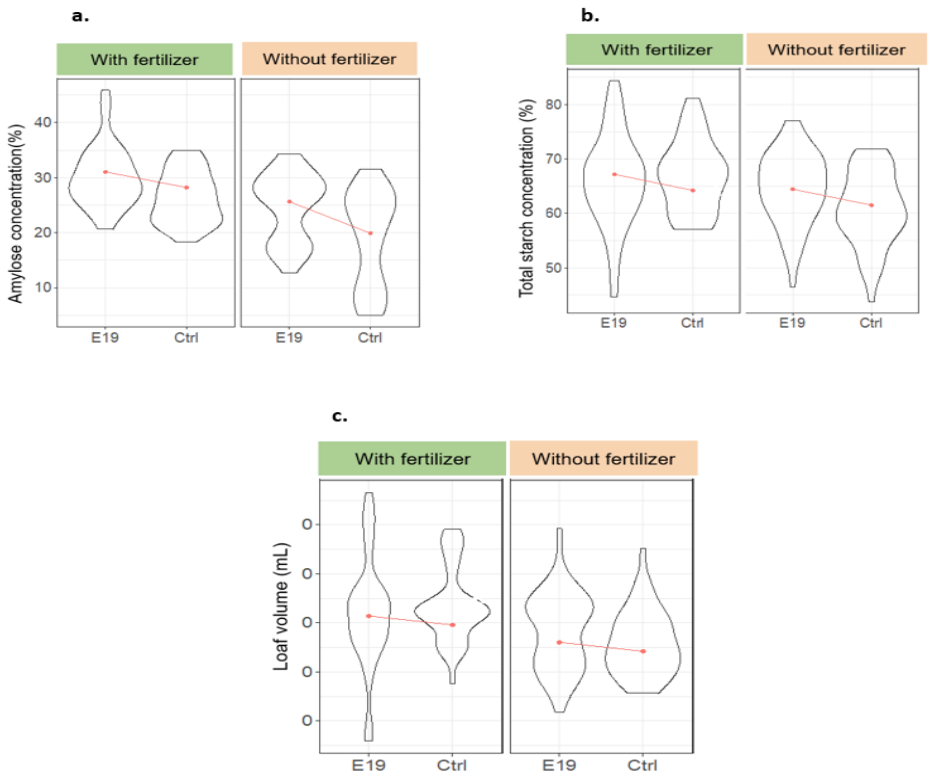

Figure 8. Linear mixed model fit by REML representing the effect of bacterial inoculation on (a) amylose concentration, and (b) total starch concentration (c) loaf volume for winter wheat based on different fertilizer management.

II.2 Wichtigste Positionen des zahlenmäßigen Nachweises

Wichtigste Position sind mit Abstand die Personalkosten der auf dem Projekt arbeitenden Wissenschaftler(in) Herr Dr. Bastian Franziski und Frau Dr. Azin Rekowski mit realen Ausgaben von ca. 160.000 €. Der nächswichtige Posten sind Sachmittel mit realen Ausgaben von ca 47.000 € im Wesentlichen für Kits, Biochemikalien und Analytik in unserer Core Facility. Dienstreisen konnten wegen der Covid Pandemie nicht alle wie geplant gemacht werden und daher entstanden hier nur Kosten von ca 5000 €. Reisekosten im Wesentlichen für Projekttreffen und Probenüberführung.

II.3 Notwendigkeit und Angemessenheit der geleisteten Arbeit

Die durchgeführten Arbeiten waren für die Aufgabenstellung des Projektes wichtig und in ihrem Umfang zur Erreichung der Projektziele notwendig

II.4 Voraussichtlicher Nutzen, insbesondere der Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

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Die in diesem Projekt erzielten Ergebnisse wurden auf verschieden nationalen und internationalen Fachtagungen der wissenschaftlichen Öffentlichkeit präsentiert und einge Teile sind bereits in wissenschaftlichen Fachzeitschriften veröffentlicht worden. Alle Daten sind über Datenbanken (Bonares, NCBI) öffentlich verfügbar. Auf den Ökofeldtagen am Gladbacherhof und in Klein Hohenheim (2022/2023) wurde das Bread&Beer Projekt auch einer interessierten Öffentlichkeit und dem Fachpublikum durch einen Stand am Versuchsfeld präsentiert. Das Projekt wurde in der BMBF Broschüre Ressource Boden vorgestellt. Die Ergebnisse wurden auf mikrobiologischen und pflanzenbaulichen Fachtagungen sowie in Vorlesungen zu Weizenqualität in unseren Modulen für MA und BA Studierende vorgestellt. In Phase II des Projekts werden die Ergebnisse zur Samenbeimpfung des Winterweizens mit *H. diazotrophicus* E19 auf weiteren Versuchsfeldern überprüft und nähergehende Untersuchungen zur Wirkung des Bakteriums auf das Wachstum von Sommergerste angestellt.

II.5 Während der Durchführung des Vorhabens dem ZE bekannt gewordener Fortschritt auf dem Geiet des Vorhabens bei anderen Stellen

Die Thematik der Ertragssteigerung und Verbesserung des Kornertrages im ökologischen Landbau ist ein wichtiges Thema, welches von verschiedenen Perspektiven und wissenschaftlichen Disziplinen bearbeitet wird. Der Einsatz von pflanzenwachstumsfördernden Bakterien ebenfalls, aber die Kombination mit der Kornqualität im ökologischen Betrieb im Feldversuch war und ist ein Alleinstellungsmerkmal dieses Projektes. In der Projektlaufzeit ist nach unserem Kenntnisstand keine Veröffentlichung erschienen, die ähnliche, für das Projekt relevante Ergebnisse publiziert hat.

II.6 Erfolgte oder geplante Veröffentlichungen

Quiroga S.**,** Ratering S.**,** Rosado-Porto D.**,** Rekowski A., Schulz F., Zörb C. und Schnell S. (2025) Seed inoculation of *Hartmannibacter diazotrophicus* does not alter the rhizosphere bacterial microbiome of wheat and barley in a three-year field trial. Appl. Soil Ecol. 206: 105823.

Quiroga S.**,** Rosado-Porto D.**,** Ratering S.**,** Rekowski A., Schulz F., Krutych M., Zörb C. und Schnell S. (2024) Long-term detection of *Hartmannibacter diazotrophicus* on winter wheat and spring barley roots under field conditions revealed positive correlations on yield parameters with the bacterium abundance. FEMS Microbiology Ecology 100: fiae023

In Vorbereitung:
Rekowski A., Wimmer M., Quiroga S., Rosado-Porto D., Ratering S., Schnell, S., und Zörb C. Seed Inoculation with *Hartmannibacter diazotrophicus*: Insights into Starch Properties and Protein Fractions in Malting Barley

Rekowski A., Wimmer M., Quiroga S., Rosado-Porto D., Ratering S., Schnell, S., und Zörb C. Enhancing winter wheat yield and bread quality Through *H. diazotrophicus* E19 Seed Coating.

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Quiroga S., Ratering S., Rosado-Porto D., Rekowski A., Schulz F., Zörb C. und Schnell, S. Distinct nucleic acids reveal differences in rhizosphere bacterial community composition of wheat and barley in a two-year field trial.

Sacharow J., Rosado-Porto D., Quiroga S., Ratering S., Geißler-Plaum R., Schneider B. und Schnell S. Which effect does the inoculation of the plant growth-promoting rhizobacterium *Hartmannibacter diazotrophicus* E19^{T} have on the microbiome of winter wheat?

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Rekowski A., Wimmer M.A., Hitzmann B., Hermannseder B., Hahn H., Zörb C. (2020) Application of urease inhibitor improves protein composition and bread-baking quality of urea fertilized winter wheat. Journal of Plant Nutrition and Soil Science 183 (2), 260-270.