



Microbial consortia and biochar as sustainable biofertilisers: Analysis of their impact on wheat growth and production

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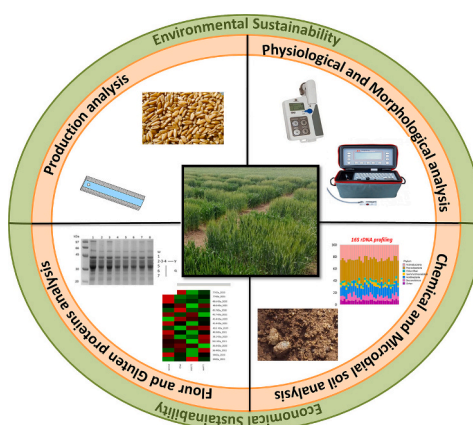
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HIGHLIGHTS

- Biofertilizers and biochar are sustainable alternatives to chemical fertilisers.
- Factors driving the most changes in wheat are the seasons climate and cultivars.
- Biofertilisers and biochar did not alter the rhizospheric soil community.
- Gliadins and LMW-glutenin subunits are modulated by biofertilisers and biochar.

GRAPHICAL ABSTRACT



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ABSTRACT

The European Union is among the top wheat producers in the world, but its productivity relies on adequate soil fertilisation. Biofertilisers, either alone or in combination with biochar, can be a preferable alternative to chemical fertilisers. However, the addition of biofertilisers, specifically plant growth promoting microbes (PGPM), could modify grain composition, and/or deteriorate the soil composition. In this study, the two wheat cultivars *Triticum aestivum* (Bramante) and *T. durum* (Svevo) were cultivated in open fields for two consecutive years in the presence of a commercial PGPM mix supplied alone or in combination with biochar. An in-depth analysis was conducted by collecting physiological and agronomic data throughout the growth period. The effects of PGPM and biochar were investigated in detail; specifically, soil chemistry and rhizosphere microbial composition were characterized, along with the treatment effects on seed storage proteins. The results demonstrated that the addition of commercial microbial consortia and biochar, alone or in combination, did not modify the rhizospheric microbial community; however, it increased grain yield, especially in the cultivar Svevo

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(increase of 6.8 %–13.6 %), even though the factors driving the most variations were associated with both climate and cultivar. The total gluten content of the flours was not affected, whereas the main effect of the treatments was a variation in gliadins and low-molecular-weight-glutenin subunits in both cultivars when treated with PGPM and biochar. This suggested improved grain quality, especially regarding the viscoelastic properties of the dough, when the filling period occurred in a dry climate. The results indicate that the application of biofertilisers and biochar may aid the effective management of sustainable wheat cultivation, to support environmental health without altering the biodiversity of the resident microbiome.

1. Introduction

Wheat ranks third in terms of total grain production after maize and rice, and the global requirement in 2023/24 was forecast at 783 million Mg (<https://www.fao.org/worldfoodsituation/csdb/en/>, data released on 30.8.2023) (Igrejas et al., 2020). The global importance of wheat is associated with its metabolic grain composition: 75 %–80 % carbohydrates (with approximately 58 % starch and approximately 13 % non-starch polysaccharides), 9 %–18 % proteins, 2 % fibres, lipids, and minerals, 0.1 % vitamins (especially B and E types), and phytochemicals (i.e. phenolic compounds, flavonoids, and carotenoids) (Wieser et al., 2020a, 2020b). Several components of wheat display a wide range of pharmacological properties, including anticancer, antimicrobial, antioxidant, and antidiabetic activities (Moshawih et al., 2022).

Wheat gluten is composed of a very heterogeneous group of proteins that can be divided into α , β , γ , and ω -gliadins, high-molecular-weight glutenins (HMW-GS), and low-molecular-weight glutenins (LMW-GS). The complex network of gliadins and glutenins imparts the unique property of viscoelasticity to dough, thus, these storage proteins have been intensively studied (Guzmán et al., 2015). Interestingly, gluten protein composition is also a bioindicator of transition agriculture (Visioli et al., 2018). Many factors modify the distribution of these compounds, for example, seeds infected with powdery mildew contain higher concentrations of glutenins and gliadins, but their ratio remains constant (Li et al., 2018); however, drought stress only increases gliadin content and heat stress decreases the LMW-GS content (Olckers et al., 2022; Phakela et al., 2021). Apart from biotic and abiotic stresses, other factors may also influence protein concentration; for example, nitrogen (N) fertilisation, the type of N, and time of fertilisation can modify the composition of protein fractions (García-Molina and Barro, 2017; Rekowski et al., 2020). In addition to the glutenins to gliadins ratio, metabolic and nutritional parameters are key characteristics influenced by the environment (i.e., fertilisation and presence of stresses) (De Santis et al., 2018; Janni et al., 2020; Zhong et al., 2018).

High temperatures, increased frequencies of heat waves, torrential rain, and long periods of drought are adverse consequences of climate change and result in reduced productivity (Bailey-Serres et al., 2019; Janni et al., 2020). Chemical treatments are frequently employed to address these issues; however, extensive use of N and phosphorus (P)-based fertilisers has various adverse effects on agricultural ecosystems, and human health, and it results in spread of inorganic and organic contaminants (Rashmi et al., 2020). To identify novel sustainable alternatives, biofertilisers have attracted attention, specifically, bacteria and/or fungi, collectively referred as Plant Growth Promoting Microbes (PGPM), which help improve plant growth, yield, and health by promoting the uptake of nutrients such as N and P by enhancing root growth and architecture (Assainar et al., 2018; Tshering et al., 2022) and potentially by strengthening the “plant immune system” (Graziano et al., 2022; Kumar et al., 2022; Tabacchioni et al., 2021; Vocciante et al., 2022). The use of PGPM can be supplemented by the simultaneous addition of soil amendments such as biochar, which is produced through heat treatment of agricultural and/or food processing waste under limited oxygen supply; a process thus supporting the “end of wastes” objective (Spani, 2020). Biochar improves soil quality and fertility by increasing water and nutrient retention and supporting soil microbial population (Gujre et al., 2021; He et al., 2021a; Marmiroli et al., 2022;

Sun et al., 2021).

Omics techniques have been applied to evaluate the effects on wheat harvested in 2020 and 2021 to different sustainable treatments (CHAR, Micosat F1, and CHAR with Micosat F1 addition). Micosat F1 is a commercial microbial consortium comprising bacteria and fungi that can solubilise P, mitigate biotic and abiotic stress, promote plant growth, and fix N (Naik et al., 2019; Tabacchioni et al., 2021). To study the effects of these treatments, physiological and agronomical data were collected during the growth period and at maturity; at the same time, the effects of the treatments were examined following various omics approaches to look at the rhizospheric soil microbiome (with regards to both fungi and bacteria), while the gluten components were analysed using a proteomic approach. This study provides new insights into the responses of wheat to various sustainable treatments, thereby promoting a holistic understanding of the effects of biostimulants on wheat.

2. Materials and methods

2.1. Plant material and growth conditions

Two wheat cultivars were utilized in this study: *Triticum durum* Desf. cultivar Svevo and *Triticum aestivum* Desf. cultivar Bramante both released by Produttori Sementi Bologna PSB s.p.a. (Bologna, Italy) in 1996 and in 2003, respectively. Svevo grain has high protein content and an extraordinary aptitude for industrial transformation and Bramante has excellent productivity, high hectolitre weight, and high resistance to Fusarium (as reported in Graziano et al., 2020 and on the PSB s.p.a. catalogue). The main features of the two cultivars are shown in Table S1. The wheat field experiments were performed in a farm (Azienda Stuard, close to the city of Parma, Italy; Lat. 44.4802300N; Long. 10.1603000E; 58 m above sea level). The agricultural soil is a silty-clay-loam soil (clay 40 %, silt 48 %, and sand 11 %), with a pH around 8, an organic content of 16.6 g kg⁻¹, and salinity of 312.5 meq 100 g⁻¹, data were analysed following standard protocols (Ministro per le Politiche Agricole, 1999) Meteorological data were collected daily by an automatic weather station installed close to the experimental field. Fig. S1B reports the rainfall distribution and maximum and minimum (over a 10-day period) mean of temperatures during the 2020 (Fig. S1A) and 2021 (Fig. S1B) cropping seasons.

Both Bramante and Svevo were sown at a density of 400 seeds m⁻² in the winter period. The experiment was set up with a split-plot design with parcels of 3 m² (eight rows, 0.20 m distance between rows and 3 m long) considering the two cultivars and the different treatments with five (2020) or three (2021) replicates for two consecutive years. The different growth conditions were: i) CTR, control condition; ii) CHAR, with biochar obtained by slow pyrolysis of wood pellet (for its full characterization see what described as biochar A4 previously (Marmiroli et al., 2018)), applied before sowing at a rate of 0.25 kg m⁻² (2.5 Mg ha⁻¹), and buried mechanically at a depth of 10 cm; iii) F1, with Micosat F1 granular microbial mix including both bacteria and fungi, provided by CCS Aosta Srl (Aosta, Italy), was applied at the ratio 1:1 w/w of seeds (which correspond to 14 g m⁻² for Bramante and 20 g m⁻² for Svevo); iv) CHAR_F1, with biochar and F1 in the same amount described above. Microbial consortia were mixed with the seeds and then delivered to the soil during sowing (see Fig. S2). The amount of seeds needed to cover one plot at the density of 400 seeds m⁻² was prepared and weighted.

Next, the same weight of Micosat F1 was added to the seeds, and the whole mix was placed in a collection chamber of the sowing machine (Fig. S2A) and distributed in 8 rows (Fig. S2B and C). Seeds and powder were visible few centimetres below the soil surface (see Fig. S2D).

The soil was fertilized with N at 50 kg ha⁻¹ just enough to replenish what had been used the year before. No potassium (K) or P was added. Consortium F1 has a specific weight of 1.127 kg L⁻¹ and is composed of: *Trichoderma harzianum* TH01 (concentration $\geq 3 \times 10^7$ spores g⁻¹, representing 0.8 % w/w of the dry product), *Glomus mosseae* GP11, *Glomus coronatum* GU53, *Glomus caledonium* GM24, *Septoglomus viscosum* GC41 (*Glomus* sp. represent 40 % w/w of the dry product), *Rhizophagus irregularis* RI31, *Bacillus amyloliquefaciens* BA41, *Pseudomonas fluorescens* PA29, *Azospirillum brasilense* AB86, *Komagataella pastoris* PP59 (all five having a concentration $\geq 6.5 \times 10^7$ spores, or CFU g⁻¹, representing 18.6 % w/w of the dry product), stabilization of the microbes was performed on zeolite.

2.2. Plant phenological analysis

Phenotypic parameters such as plant height, spike length, and biomass, were measured on three plants taken from each plot. Biomass yield of each plant was measured separately from the dry weight (DW) of the shoots at the end of the vegetative cycle in June. Plant tissues were dried out in an oven at 70 °C for 24 h and the weight of each single plant was measured. Chlorophyll content was evaluated using the portable Chlorophyll Meter SPAD-502 plus (Konika Minolta, Europe GmbH) at booting and flowering periods, leaf transpiration resistance, LTR, was measured by AP4 Porometer (Delta-T Device, Lombard & Marozzini SRL, Rome, Italy). Recorded data include grain yield (GY) (kg ha⁻¹), thousand kernel weight (TKW) (g), and test weight (TW) (kg hL⁻¹). TKW was obtained as the mean value of 3 replicates of 100 seeds from each plot. TW was calculated using a Shopper chondrometer equipped with a 1 L container and reported as kg hL⁻¹ (Rieger GmbH, Austria).

2.3. Soil chemical and biological analysis

Soil samples were collected according to the table reported in the Supplementary data (see Table S.2).

Chemical analysis. Samples of bulk soil (around 0.5 kg) from each plot were collected at 10 cm depth. Each sample was air-dried and stored at room temperature, while the rhizospheric soil (attached to roots) was collected and immediately stored at -80 °C. Soil pH and Electrical Conductivity (EC) (Mettler-Toledo S.p.A., Milan, Italy) were measured by performing potentiometric measurements in a 1:2.5 soil-water suspension as reported previously (Ministro per le Politiche Agricole, 1999). The chemical analyses of organic carbon (C) and N were performed according to standardized protocols (Ministro per le Politiche Agricole, 1999). Determination of P, calcium (Ca), iron (Fe), zinc (Zn), and K concentrations were carried out by Inductively Coupled Plasma Mass Spectrometry (MS) using the iCAP™ TQe ICP-MS (Thermo Fisher Scientific Inc., Waltham, MA, USA) following standard protocols (International Organization for Standardization (ISO), 2016; UNI.EN, 2012).

Biological analysis. Five g of rhizospheric soil samples were collected for each growth condition and each plot, and stored at -80 °C. Metabarcoding analysis of microbial DNA from rhizospheric soil was performed as described (Graziano et al., 2022). Briefly, genomic DNA was isolated from 250 mg of rhizospheric soil using NucleoSpin® Soil (Macherey-Nagel, Duren, Germany). 50 ng of DNA was used from each sample to amplify the genes encoding 16S rRNA (V3-V4 region), and 18S (ITS2 region) as reported previously (Graziano et al., 2022; Takahashi et al., 2014; White et al., 1990). Amplification followed a standard protocol (Graziano et al., 2022) and amplicons were subjected to Next Generation Sequencing (Basciani et al., 2020; Juhmani et al., 2020) (BMR Genomics Srl, Padua, Italy).

2.4. HMW-GS genotype of Bramante

The allelic structure at the *Glu1* locus for HMW glutenins in Svevo was already known from literature studies (Graziano et al., 2020); but was unknown in Bramante. The allelic composition at the *Glu1* locus was obtained through genomic, and proteomic analysis (in both cases details are given in Supplementary Material paragraph “HMW-GS genotype of Bramante”).

2.5. Extraction, separation, and characterization of seed storage proteins

Wheat seeds from each cultivar (35 g of samples) were milled with Knifetec 1095 (Foss, Hillerød, Denmark) to produce a fine powder of whole-meal semolina. Gluten proteins (gliadins, HMW-GS, and LMW-GS) were extracted from wheat semolina (30 mg) as previously described (Graziano et al., 2019) and their concentration was measured by Bradford assay (Bio-Rad, Hercules, CA, USA). Total N in the flour was determined by Kjeldahl method (Ministro per le Politiche Agricole, 1999). The proteins fractions were separated by SDS-PAGE followed by densitometric analysis, to obtain a relative quantification of the protein bands, as previously described (Graziano et al., 2019). Gliadins were subdivided into four classes (ω , α , γ , and β), similarly, LMW-GS were subdivided into 42, 37, 32, and 31 kDa, as previously reported (Graziano et al., 2019). Relative amounts of sub-fractions were calculated by considering all bands present in each lane. Three replicates were performed for each fraction. Protein bands, identified as differently abundant, were then taken from the polyacrylamide gel, digested, and prepared for mass spectrometry (MS)-based characterization (Shevchenko et al., 2007). In particular, samples were run on a HPLC-LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA), and the raw data were analysed using the *Viridiplantae* proteomic databases (Graziano et al., 2019).

2.6. Statistical analysis

Statistical analysis was performed using the statistical package SPSS (v25.0) for Windows (© Copyright IBM Corporation 1989, 2012) or Past 4.03 (© Copyright Hammer 1999–2020). Phenotypic data are presented as mean \pm SD and were analysed by ANOVA, non-parametric Kruskal-Wallis test with Bonferroni correction (significance level of $p = 0.05$). The storage proteins amount was evaluated by densitometric analysis, on three replicates, using the Image Lab 4.5.1 software (Bio-Rad, Hercules, CA, USA). Mean values were compared by ANOVA followed by the post-hoc Dunn's test. p -value ≤ 0.05 was considered statistically significant. The amounts of the different gliadins, LMW-GS, and HMW-GS sub-units, which were calculated as reported above, are shown as heat maps, which were obtained using the Heatmapper software (<http://www.heatmapper.ca/>). For metagenomic data, PERMANOVA results were calculated with Bray-Curtis as a distance metric.

3. Results and discussion

3.1. Environmental conditions in the two growing seasons

The two growing seasons had different weather conditions (Fig. S1). From February 2020, during which temperatures started to increase and wheat growth was initiated, the total rainfall was 186 mm, and the mean air temperature was 14.4 °C (min -3.5 °C -max 33.5 °C). In 2021, the total rainfall was 150 mm, and the mean air temperature was 12.5 °C (min -6.9 °C -max 36.2 °C). High temperature during seed development is known to constitute a stress affecting the duration and rate of the grain-filling period (Flagella et al., 2010; Zingale et al., 2023). Water availability during vegetative growth determines the pattern of plant development; however, excess water during this same period reduces the amount of gluten protein (Graziano et al., 2019; Zingale et al., 2023).

3.2. Results of field trials performed in two seasons

Analysis of production traits and plant biomass of cultivars Svevo and Bramante (Fig. 1 and Table S3) in 2020 showed that grain yield was in line with the average yield observed for both durum and bread wheat during the same season in Italy (Regione Emilia-Romagna, 2021). In 2021, the yields of both cultivars were in line with the production amount registered in the Region Emilia-Romagna in the same year, as indicated by the Italian Agriculture Consortia (<https://www.consorziagr.ariditalia.it/>) and (Regione Emilia-Romagna, 2022), with a 20 % increase compared with the previous season. For both seasons, supplementation with consortium F1 elicited a grain yield increase in Svevo, i.e. 6.84 %–13.64 % (Fig. 1 and Table S3), while the CHAR treatment determined an increase of 6.72 %–16.06 %. Cultivar Svevo has been reported to exhibit improved N use efficiency under conventional N and water regimes (Lupini et al., 2021), good drought resistance (Gulli et al., 2022), and to be positively affected by the presence of arbuscular mycorrhizal fungi (Fiorilli et al., 2022). Biochar improves water and nutrient retention as well as soil quality and fertility (Ding et al., 2016; Igalavithana et al., 2015). PGPMs can fix N and solubilise P, thereby making these nutrients available to plants. Moreover, PGPMs regulate phytohormone production and promote plant tolerance to abiotic stress by increasing the antioxidant levels (Lopes et al., 2021; Naik et al., 2019). Considering that in Svevo the final yield under PGPM treatment was higher than that in the control condition, it can be stated that both biochar and PGPMs can provide positive stimuli for plant growth.

In Bramante, an increase in yield was observed only in 2020 when the soil was supplemented with F1 and CHAR (Fig. 1 and Table S3). This may also be attributed to the different climates in 2021 during the critical heading and flowering stages of Bramante (Mäkinen et al., 2018; Sadeghi-Tehran et al., 2017). These stages had different seasonal distributions in Svevo, which entered the heading and flowering stages earlier than Bramante. Principal component analysis (PCA) of the data from 2020 and 2021 for both cultivars showed grouping depending on the season and the cultivar (Fig. 2A), which implied that the two climate parameters (i.e. temperature and rain) and cultivar were the factors explaining more of the variance than the treatments. Similar results

were obtained by network analysis (Fig. 2B). Nodes belonging to the same season and cultivar clustered together, with stronger connections between samples belonging to the same cultivar and grown in the same season (Fig. 2B). In the PCA, PC1 explained 58.2 % of the variance and was positively correlated with TKW, shoot dry biomass and plant height. Plant height is strongly regulated by genetics (Würschum et al., 2015) and very little variation is expected with one cultivar, regardless of the growing conditions. The pattern of shoot-dry biomass variation followed that of the increase in yield in 2021. TKW is an important component of yield with a complex genetic basis determined by grain width, length, thickness, shape, and density (Cao et al., 2020; Ji et al., 2023; Liu et al., 2020). Moreover, larger seeds are known to result in better seedling vigour, a trait that is positively correlated with yield and is expected to increase flour yield (Farahani et al., 2011). For TKW, positive variation was observed in both cultivars treated with CHAR or F1 in 2020. A more comprehensive overview of the content of Svevo grains has been produced recently using metabolomics. Data showed biochar and F1 treatment increased the production of lipids (particularly affecting the glycolipid desaturation pathway), and flavonoids, whereas that of carbohydrates was reduced (Riboni et al., 2023). These modifications exert multiple positive effects. Glycolipids are involved in the synthesis of the hormone jasmonic acid, a metabolite that regulates growth and development and plays a role in plant stress responses (Li et al., 2022). Moreover, upregulation of these metabolites improves the quality of flour and loaf volume after fermentation (Min et al., 2020). Increased flavonoid content may occur in plants in response to biotic and abiotic stressors (De la Rosa et al., 2009; EL Sabagh et al., 2021). In addition, these compounds are recognised for their antioxidant and beneficial properties in the treatment of various diseases (Shen et al., 2022; Shewry and Hey, 2015). Finally, a reduction in small fermentable carbohydrates can be beneficial to people affected by irritable bowel or inflammatory bowel syndrome (Shepherd, 2014).

PC2 explained 27.7 % of the variance and it was positively correlated with changes in chlorophyll content measured during the various growth stages (Fig. 2A and Table S3). Indeed, Svevo, when treated with F1, CHAR, or a combination of the two, displayed an increase in this physiological trait (Table S3) during both seasons (range of variation 3

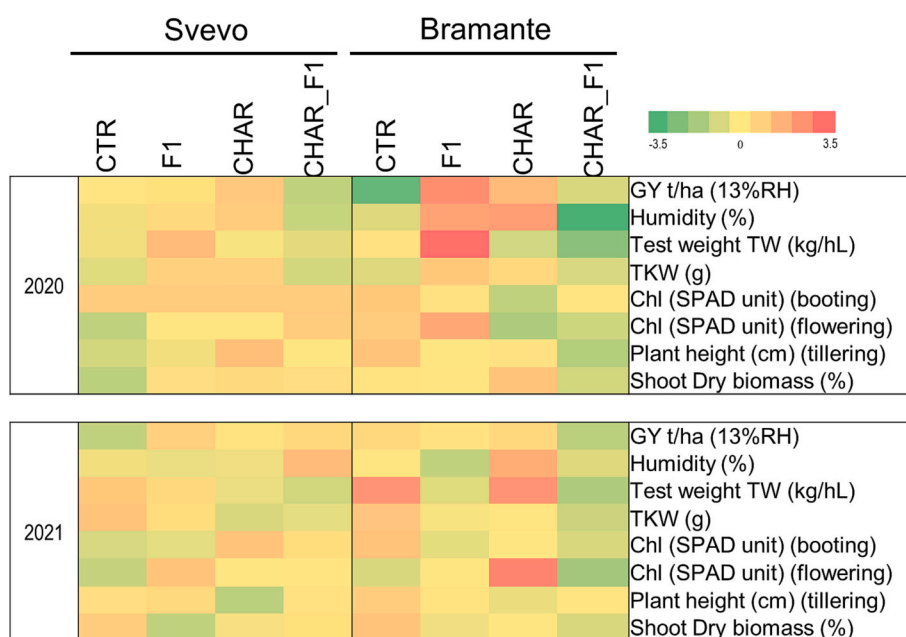


Fig. 1. Heatmaps of physiological data from the 2020 and 2021 seasons for both cultivars Svevo and Bramante. Data were standardized (standardized data were calculated as: measure – average/standard deviation), traits decreasing with respect to the average measurements are in green, while traits increasing correspond to a scale of orange-red, as reported in the figure. The treatments are reported as: CTR: control, F1: Micosat F1, CHAR: biochar, and CHAR_F1: biochar with Micosat F1. Full data are available in Table S3.

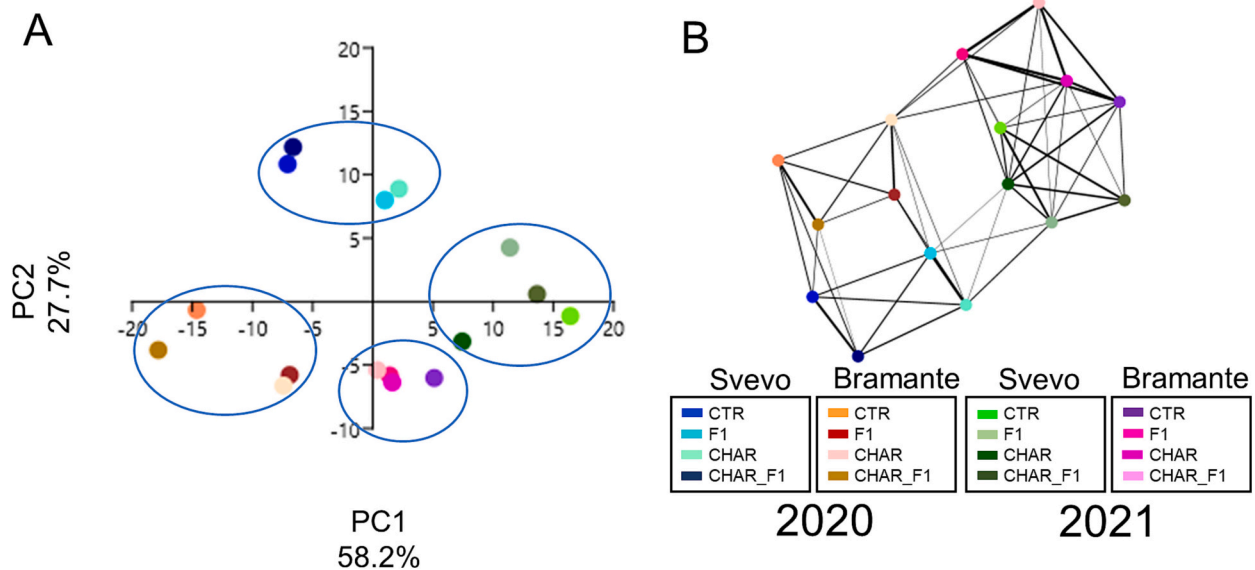


Fig. 2. Principal component analysis (PCA) of physiological data (fully reported in Table S3) in two cultivars (*T. aestivum* cv Bramante and *T. durum* cv Svevo) throughout two growing seasons, 2020 and 2021; (A); network analysis of physiological data (B). In both cases, samples were colour-coded according to the legend presented below in panel B. The treatments are reported as: CTR: control, F1: Micosat F1, CHAR: biochar, and CHAR_F1: biochar with Micosat F1.

% - 8 %), suggesting stimulation of photosynthesis, which is related to yield increase (Long et al., 2006; Makino, 2011), as was observed (Table S3), this may be attributed to the PGPM treatment. Indeed, some of the bacteria present in Micosat F1 produce siderophores, i.e. compounds that render Fe^{3+} bioavailable to plants; this elemental is involved in the biosynthesis of chlorophyll and it is found in the iron-containing (haeme) proteins of plants, such as cytochromes, which are important members of the electron transfer systems in chloroplasts. These are some of the activities that help define a PGPM as such (Lopes et al., 2021; Naik et al., 2019; Yadav et al., 2022). Moreover, the presence of biochar can help retain water, and nutrients, making them available to crops (He et al., 2021b; Sun et al., 2021).

Before harvesting, the plants were examined for morphological damage caused by abiotic or biotic stress. No cold or lodging damages were observed. No traces of *Septoria*, *Fusarium* (head blight), *Puccinia* (brown rust), or powdery mildew, were detected in any plot. Deoxynivalenol was measured using a commercial ELISA on the wheat flours (Svevo and Bramante) from the 2020 and 2021 field trials, but was not detected in any of the samples (data not shown).

3.3. Soil chemical composition

Soil plays a crucial role in plant growth and food production, and it constitutes the basis for >95 % of global food production. Macronutrients (P, N, Ca, K, and Mg) and micronutrients (Zn, Fe, and Mn) are essential for crop production and their concentrations are crucial in determining soil health. Soil microorganisms can influence the concentrations of some nutrients as they can fix N and solubilise P, K, and Zn (Gruber, 2015). Therefore, the concentration of organic C, total N, P, Ca, Fe, Zn, and K were determined for each treatment and year. Soil was collected at the plant maturity stage to evaluate whether yield (increase or loss) was related to soil quality. The results (Table S4) showed that the concentrations of these elements were within the respective average range for healthy soil (Noulas et al., 2018; Pratim et al., 2022; Shiwakoti et al., 2020, 2019). No differences were observed between treatments or cultivars but between growing seasons. In 2020, the soil Ca content was lower than that in 2021, and Fe, Zn, and K contents were lower in 2021 (Tables S4 and S5). Nevertheless, all values remained within the average range. The pH was also slightly higher in 2020 than in 2021. Moreover, at the concentration of biochar added during the experiments, no

significant increase in pH was observed. This may be related to the fact that the soil pH (7.8–8.6) and biochar pH (8–8.2, see biochar A4 in Marmioli et al., 2018) were relatively similar. Moreover, the quantity of biochar used in the fields represented approximately 0.05 % of the topsoil C.

3.4. Rhizospheric soil analysis

3.4.1. Rhizospheric bacterial population

Rhizosphere samples from Svevo and Bramante were collected in May and June 2020. At both time points, the phyla and classes were analysed in detail. The most abundant phyla (percentages are rounded values; Fig. 3A and Fig. S3) were: Proteobacteria (37 %), Actinobacteria (24 %), Acidobacteria (16 %), Bacteroidetes (9 %), Gemmatimonadetes (3 %), Chloroflexi (3.5 %), Verrucomicrobia (26 %) and TM7 (0.7 %). The most variable populations were Bacteroidetes, Verrucomicrobia, and TM7 in Bramante and Bacteroidetes, Gemmatimonadetes, and TM7 in Svevo. A PCA did not show any clustering, and a PERMANOVA did not show any significant differences between the sampling times, cultivars or treatment. The taxa with the most pronounced variance were Actinobacteria, which positively correlated with changes in PC1, and Bacteroidetes, which correlated with variations in PC2 (Fig. S4A). Actinobacteria is a very diverse bacterial phylum in which *Streptomyces*, *Nocardiopsis*, and *Arthrobacter* are the most common genera. In our samples, *S. mirabilis* represented 1 % of the total population, and this bacterium forms biominerals (Yang et al., 2012). Strains belonging to the Actinobacteria group can solubilise K and P to act as PGPM, biocontrol agents and increase plant resilience to abiotic stress (van Bergeijk et al., 2020). Indeed, Actinobacteria and Bacilli, can avoid drought stress by assuming dormancy and resurrecting when environmental conditions improve (Bouskill et al., 2016; Naylor et al., 2017). Their positive effects on crop yield have frequently been reported (Boukhatem et al., 2022; Sansinenea, 2019). Bacteroidetes are involved in the degradation of complex organic matter, and their abundance is an indicator of agricultural soil usage (Wolińska et al., 2017). The most abundant classes (Fig. 3B, percentages are rounded values) were: Alphaproteobacteria (22 %), Actinobacteria (14 %), Acidobacteria-6 (8 %), Betaproteobacteria (6 %), [Chloracidobacteria] (6 %), Gammaproteobacteria (6 %), Thermoleophilia (4 %), and Sphingobacteria (1 %). The high abundance of Alphaproteobacteria was expected, because

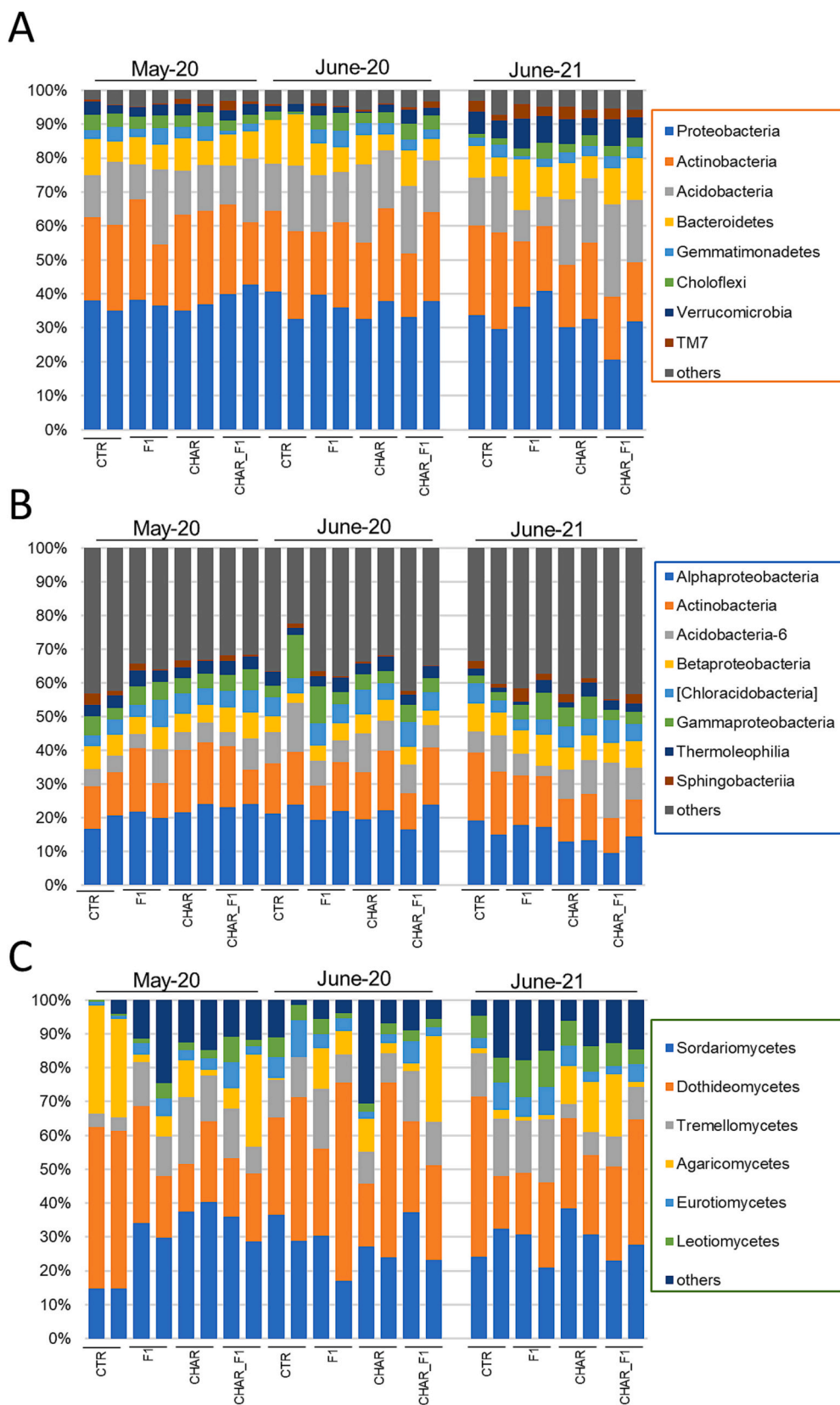


Fig. 3. Rhizospheric bacterial communities at the level of phyla (A) and classes (B) and rhizospheric fungal communities at the level of classes (C), collected from Svevo' soils of plants untreated (CTR) or treated with Micosat F1 (F1), biochar (CHAR), or biochar with Micosat F1 (CHAR_F1). (A), (B) and (C) report the relative abundance (based on OTUs) of the most abundant phyla or classes found in the rhizospheric soil of wheat in duplicate and collected in May and June 2020 and in June 2021. The colours are ordered according to the legend reported at the right of the image.

together with Beta- and Gammaproteobacteria, this class belongs to the phylum Proteobacteria (Spain et al., 2009), which was the most abundant phylum in our soil samples.

This analysis was repeated in 2021; however, only one-time point, i. e. June, was considered. No significant differences were observed between the two sampling periods during the previous year. When microbes are added to the soil, modifications of the rhizospheric microbiome may only persist for a short period of time, as previously suggested (Fierer, 2017), unless the microorganisms can survive and outgrow the previously resident community. In 2021, the distributions of the phyla and classes were similar to those observed in 2020 (Fig. 3A and B). The PERMANOVA did not reveal any differences between cultivars, treatments or years. A PCA of the 2021 microbial population did not produce any grouping (Fig. S4B). Acidobacteria were the main components correlated with variance in PC1, displaying a large repertoire of genes responsible for regulating several metabolic functions (i.e. N, S, carbohydrate metabolism, cellulose, and siderophore synthesis) (Kalam et al., 2020). In contrast, Bacteroidetes were positively correlated with changes in PC2 (Fig. S4B). α -Analysis (Table S6) revealed a Shannon diversity index of approximately 4.4 for all soil samples and a Chao-I richness index of approximately 170 in the rhizospheric populations of Bramante and Svevo. None of the treatments significantly changed the diversity or richness indexes of microbial populations.

3.4.2. Rhizospheric fungal population

Fungal phyla were dominated by Ascomycota and Basidiomycota, representing approximately 80 % and 10 % of the phyla, respectively (data not shown), while the most abundant class was Sordariomycetes (approximately 30 % of all classes), followed by Dothideomycetes, Tremellomycetes, Agaricomycetes, Eurotiomycetes, and Leotiomycetes (Fig. 3C and S3C). The first classes are the most representative of agricultural soils (Dang et al., 2018), and the last three classes exhibited variable proportions; however, statistical analysis did not indicate any correlation with the treatment. Significant differences were observed when the samples were grouped according to season and cultivar. Indeed, by comparing these four groups using PERMANOVA (see the table in Fig. S5), significant differences were evident between the cultivars in 2020 and between the two seasons for Bramante (Fig. S5). Variation in PC1 was positively correlated with Dothideomycetes, whereas that in PC2 was positively correlated with Agaricomycetes. Taxa belonging to the Agaromycetes can recycle organic matter because their genome contains many genes encoding hydrolytic and oxidative enzymes for lignocellulose; moreover, Agaricomycetes can degrade hydrocarbons in the soil (Mohammadi-Sichani et al., 2017; Ruiz-Dueñas

et al., 2021). The Dothideomycetes group contains a wide range of fungal decomposers of wood and is involved in biogeochemical cycles (Hyde et al., 2013). This was linked to a significant difference in the total number of fungi present in the soil, which was lower in 2021, as indicated by the Shannon diversity and Chao-I richness indexes (Table S6). Fungal abundance and richness are strongly associated with climate conditions (Bakar et al., 2020; Marčiulytė et al., 2022). Therefore, to improve soil health, it is important to define the weather conditions that promote the growth of favourable fungi and to identify all the elements that can promote the growth of the taxa that are considered soil pre-biotics (Du et al., 2021).

3.5. Gluten protein content

Gliadins were the most abundant component of the gluten fraction followed by LMW-GS and HMW-GS. The amounts of each fraction in the investigated samples are listed in Table 1.

Gliadin content was significantly lower ($p < 0.05$) in 2020 (53.61 % in Svevo and 53.06 % in Bramante) than in 2021 (62.92 % in Svevo and 69.84 % in Bramante), regardless of the treatment. In 2020, treatment with F1 resulted in a significant increase in gliadins compared to the control, but only in Bramante. In 2021, the amount was higher in both cultivars under the control conditions than in the treatments. Regarding the LMW-GS content, only for Svevo, there was a significant ($p < 0.05$) increase with the F1 treatment in 2020, while in 2021, a significant increase was observed in Svevo with the combination of CHAR and F1 ($p < 0.01$), compared to the control.

The amount of HMW-GS was higher in 2020 than in 2021 for all treatments in both cultivars. In 2020, no difference was observed between treatments, whereas in 2021, Svevo cultivated with CHAR as well as with F1 showed higher HMW-GS amounts, as did Bramante treated with F1, compared to the control.

Although the HMW-GS fraction only accounts for up to 10 % of total flour protein, it affects gluten viscoelastic properties (Graziano et al., 2020). In Svevo, the HMW-GS allele combination of Bx7 and By8 is important for dough quality and loaf volume. Similarly, in Bramante, the Glu-Ax2*, Glu-Dx5, and Glu-Dy10 alleles confer excellent viscoelastic properties to flour (Gale, 2005). The N content, determined using the Kjeldahl method, and the glutenin/gliadin (GLU/GLI) and the HMW-GS/LMW-GS ratio, are important parameters for technological quality, which are shown in Table 1. The differences in the abundance of the gluten fractions and the corresponding GLU/GLI and HMW-GS/LMW-GS ratios differ significantly between both treatments and seasons (Table 1). The GLU/GLI ratio was higher in 2020 than in 2021, whereas

Table 1

Reserve proteome composition of gliadins and glutenins fractions of Svevo and Bramante cultivated in 2020 and 2021. The treatments are reported as: CTR: control, F1: Micosat F1, CHAR: biochar, and CHAR_F1: biochar with Micosat F1.

Year	Cultivar	Treatment	%N	GLI%	LMW%	HMW%	GLU/GLI	HMW/LMW		
2020	Svevo	CTR	5.92	52.97 ^a	20.88 ^b	26.16 ^a	0.89 ^a	1.25 ^a		
		CHAR	9.63	53.64 ^a	20.20 ^b	26.16 ^a	0.86 ^a	1.30^a		
		F1	10.54	52.78 ^a	21.65^a	25.57 ^a	0.89 ^a	1.18 ^b		
		CHAR_F1	6.78	53.37 ^a	20.69 ^b	25.94 ^a	0.87 ^a	1.25 ^a		
	Bramante	CTR	12.36	51.80 ^b	20.19 ^a	28.02 ^a	0.93 ^a	1.39 ^a		
		CHAR	10.37	53.61 ^{ab}	18.79 ^a	27.60 ^a	0.87 ^a	1.47 ^a		
		F1	10.94	53.73^a	19.08 ^a	27.19 ^a	0.86 ^a	1.42 ^a		
		CHAR_F1	12.36	53.10 ^b	19.85 ^a	27.05 ^a	0.88 ^a	1.36 ^a		
		2021	Svevo	CTR	11.45	66.12^a	20.87 ^{ab}	13.01 ^b	0.51 ^b	0.62 ^b
				CHAR	11.62	63.20 ^b	20.25 ^{ab}	16.55 ^{ab}	0.58 ^b	0.82 ^{ab}
F1	11.57			65.67 ^{ab}	17.39 ^b	16.94 ^{ab}	0.52 ^b	0.97^a		
CHAR_F1	10.94			56.72 ^b	24.71^a	18.57^a	0.76^a	0.75 ^{ab}		
Bramante	CTR		11.17	71.89^a	16.25 ^b	11.86 ^b	0.39 ^a	0.73 ^b		
	CHAR		10.48	69.45 ^b	17.60 ^{ab}	12.94 ^{ab}	0.44 ^a	0.74 ^b		
	F1		10.20	67.92 ^b	16.99 ^{ab}	15.09^a	0.47 ^a	0.89^a		
	CHAR_F1		10.43	70.14 ^b	18.40^a	11.46 ^b	0.43 ^a	0.62 ^b		

Different letters in the same column, and in each group of data, correspond to statistically different values ($p < 0.05$ one-way ANOVA, post hoc Dunn's test). Values bold-faced are significantly different.

the HMW-GS/LMW-GS ratio was decreased in 2020. In 2021, during the grain-filling period, plants experienced water scarcity combined with high temperatures, which is a condition influencing gluten quality and coeliac toxicity (Manfredi et al., 2015; Ronga et al., 2020). Interestingly, Svevo, treated with CHAR and F1 showed a significant increase in HMW and LMW glutenins and a decrease in gliadins, which may have positively influenced gluten quality.

In developing grains, the accumulation of certain protein fractions is highly ordered (Daniel and Triboi, 2001), and gliadins are rapidly produced during the middle growth period of the wheat kernel. Subsequently, glutenin accumulates in smaller quantities until the last half of the grain-filling cycle and are measurable approximately 20 days after anthesis (Phakela et al., 2021). Growth and environmental conditions during grain-filling affect protein quality, particularly with regards to kernel size and composition (Graziano et al., 2020). Studies have shown that the agronomic management can affect grain yield, protein concentration, and composition (Pichereaux et al., 2019; Zingale et al., 2023). Therefore, the amount of each gluten protein fraction was compared between the Svevo and Bramante cultivars in both seasons. We observed variations in gluten protein abundance, in particular, an increase in gliadins and a decrease in glutenins in 2021 compared to 2020, which can be attributed more to environmental factors (season) than to treatment (Table 1) (Phakela et al., 2021; Ronga et al., 2020). Considering the treatments, the results suggested that in 2021 (less rain during the grain filling period), treatment with CHAR and F1 resulted in

a significant increase in the LMW-GS fraction in both cultivars.

3.6. Identification and quantification of gluten protein subunits

The gluten fractions of the two groups were analysed using SDS-PAGE. Fig. S6 shows examples of the profiles of gliadins, LMW-GS, and HMW-GS. These protein profiles were maintained in both cultivars, albeit with some differences in the relative abundances of gliadins and LMW-GS between cultivars, years, and treatments, as shown by the densitometric analysis of electrophoretic gels (Fig. S6; Table S7).

3.6.1. Gliadins variations in Svevo and Bramante

The gliadin pattern of wheat is characterised by ω -gliadins in the 66–55 kDa range and α , β , and γ -gliadins in the 44–33 kDa range (bands 1 to 7) both in Bramante (Fig. 4A) and in Svevo (Fig. 4B). The proteins were identified by MS (Table S8). Densitometric analysis of gliadins in Bramante (Fig. 4A) and Svevo (Fig. 4B) showed that the main differences occurred between years. Environmental factors may have strong effects on gliadin components (both α - and γ -type) (Ronga et al., 2020). Our results confirmed these trends. In Bramante, GLY-48.6, GLY-45.7, GLY-43.4, and GLY-40.3 kDa were more abundant in 2020 than in 2021 ($p < 0.05$), whereas GLY-38.1 and GLY-34 kDa, were more abundant in 2021 than in 2020 ($p < 0.05$). No significant variation between years was observed for the subunits GLY-77 and GLY-36.4 kDa. In Svevo, GLY-60, GLY-44, GLY-41, and GLY-32 kDa were more abundant in 2020 than in

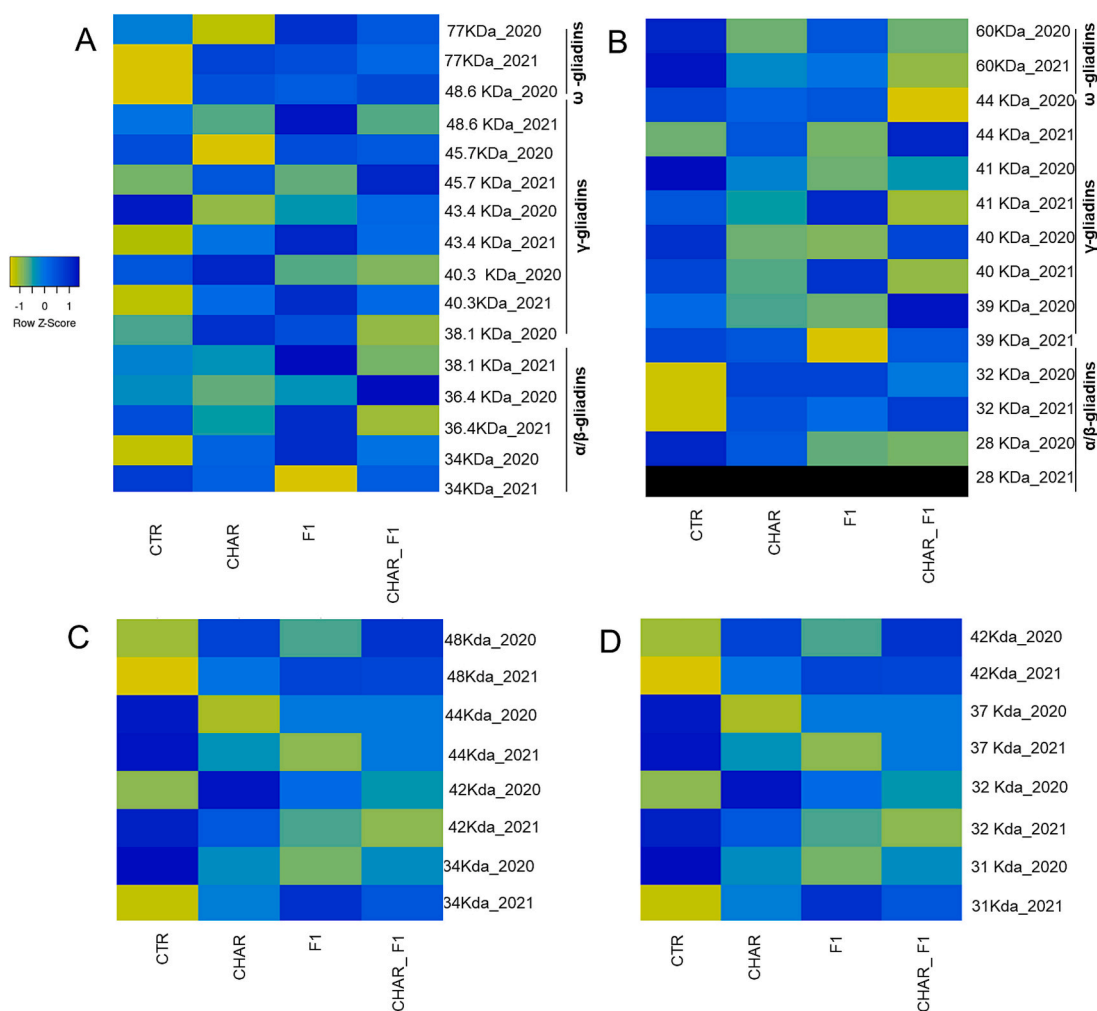


Fig. 4. Heat map representing the relative abundance evaluated by densitometric analysis of gliadins and LMW-GS purified from flours of Bramante (A-C) and Svevo (B-D) cultivated in 2020 and 2021 with different treatments (SDS-PAGE images are reported in Fig. S6). The treatments are reported as: CTR: control, F1: Micosat F1, CHAR: biochar, and CHAR_F1: biochar with Micosat F1.

2021; GLY-40 and GLY-39 kDa were more abundant in 2021 than in 2020, and GLY-28 kDa was present only in 2020. Regarding the differences between treatments, in 2021, the most significant ($p < 0.05$) variations were identified when plants were cultivated with the combined treatment of CHAR and F1. Indeed, compared with the control, the amount of GLY-40 was reduced, whereas that of GLY-32 kDa was increased (Table S6).

3.6.2. Glutenins (LMW and HMW-GS) variations in Svevo and Bramante

The LMW-GS (locus *Glu-B3*) pattern in Bramante (Fig. 4C) showed four main bands in the range of 48–34 kDa, whereas the LMW-GS pattern in Svevo (locus *Glu-B3*) was represented by one main band of 42 kDa and three minor bands (37, 32, 31 kDa; LMW-2 profile) to be seen at Table S7 and Fig. 4D. Densitometric analysis of LMW-GS (Fig. S6) in both cultivars showed significant variation ($p < 0.05$) with regards to the subunits, mainly in terms of the year of cultivation and, in some cases, treatment. In Bramante, LMW-44 and LMW-34 kDa were more abundant in 2020 than in 2021 for all treatments. In 2021, the LMW-42 kDa form was less abundant ($p < 0.05$) in the treatments CHAR_F1 and CHAR alone. Indeed, LMW-42 kDa has a higher molecular weight than the other LMW-GS subunits, presumably because of a larger number of repeats units. These longer repeat regions enhance dough strength and elasticity compared with subunits with fewer repeated-sequence domains (Masci et al., 1998). The results suggest that in wheat, the treatment with biofertilisers and biochar determines a variation in the grain flour that may affect the viscoelastic properties of the dough.

Regarding LMW-GS in Svevo, the effect of the weather conditions of the two years was pronounced, whereas that of the treatments used was not. LMW-42 kDa was more abundant in 2020 than in 2021 in all treatments, while LMW-37, 32, and 31 kDa were more abundant in 2021 than in 2020. In addition, LMW-31 kDa was absent in the control and F1 treatments in 2021. The HMW-GS profile of Svevo (locus *Glu-B1*) was represented by a band of 86 kDa (Bx7) and by a band of 74 kDa with two isoforms (By8; Fig. S6). This allelic combination is associated with favourable dough properties. The Bramante HMW-GS profile (Fig. S6F) was composed of five main bands (2*, 7, 8, 5, and 10) identified by comparing the results obtained with other wheat cultivars, with known allelic profiles using PCR and proteomics. Densitometric analysis of HMW-GS in both cultivars under the different treatments in both years did not show any significant differences (data not shown).

The accumulation of gluten proteins is a complex process affected by environmental and abiotic signalling. Environmental conditions determine changes in the abundance of gluten fractions; high temperatures exert a significant effect on the temporal regulation of genes affecting the transcription of gliadin, and glutenin genes, thereby altering the GLU/GLI ratio and ultimately affecting dough properties (Altenbach et al., 2002).

3.7. Environmental and economic considerations

Environmental sciences are in close continuity with food-producing networks when anthropized environments, such as agri-food ecosystems, are considered. These systems consume large amounts of water, energy, and chemicals, leading to poor sustainability. The output of greenhouse gases (GHGs), such as CO₂, CH₄, and N₂O, makes agroecosystems even more unbalanced, and as such, they contribute to 31 % of human-caused GHG emissions (FAO, 2021). Chemical fertilisers and pesticides are among the major contributors to these emissions (Savci, 2012a, 2012b).

Life cycle analysis (LCA) is frequently used to examine the contribution to GHG emission during the production of commodities. The dimensions of the field trials presented in this study did not provide the possibility of performing an LCA with sufficient confidence levels; however, the results presented in this study and those available in the literature can be used to assess conventional systems (scenario #1: chemical fertilisers plus pesticides and insecticides), and a possible

transition to PGPMs with or without biochar and to describe the differences based on their carbon footprint (CF) (Fig. S7). The CF of wheat is reported to be 3 Mg CO₂eq ha⁻¹ (and 0.361 Mg CO₂eq Mg⁻¹ of grain), of which 60 % (1.8 Mg CO₂eq ha⁻¹) is attributable to the use of fertilisers and 7 % (0.21 Mg CO₂eq ha⁻¹) to the spread of pesticides and insecticides (Gan et al., 2011; HGCA, 2012; Hou et al., 2021; Kashyap and Agarwal, 2021; Tubiello et al., 2021), while the remaining 1 Mg CO₂eq ha⁻¹ includes tillage, sowing, and harvesting, or use of farm machinery. Substituting chemical fertilisers with PGPMs the latter at 10 kg ha⁻¹ (scenario #2), as suggested by the manufacturer (although in our study concentrations were higher owing to the geometry of the field trials), the total output is 1 Mg CO₂eq ha⁻¹ (Tensi et al., 2023), which includes the costs of producing PGPMs (Fig. S7). The use of PGPMs instead of chemical fertilisers reduced the output of CO₂ by 1 Mg CO₂eq ha⁻¹, which can be further reduced by avoiding pesticides and insecticides. According to the literature, avoiding pesticides and insecticides will provide an extra saving of 10 %–20 % (Tensi et al., 2023); and in the field trials described here, no such compounds were used and no adverse consequences in terms of plant health or grain yield were observed. However, the largest difference in CF was obtained when considering the simultaneous addition of biochar to the soil (scenario #3), which also elicited a better plant response in terms of growth. Biochar stands for a negative output ranging from –0.47–25 Mg CO₂eq ha⁻¹ depending on the applied amount, the raw material, and the employed technology (Brassard et al., 2018; Hamedani et al., 2019). In the current study, biochar was produced from wood chips by slow pyrolysis and was supplied at 2.5 Mg ha⁻¹, which resulted in a CF of –10 Mg CO₂eq ha⁻¹. Combining the two calculations (PGPMs and biochar), the total saving was –9 Mg CO₂eq ha⁻¹, which resulted in a global saving of –11 Mg CO₂eq ha⁻¹ (Fig. S7). This simulation did not consider the CO₂ input into the residual biomass (straw) and grain, which is approximately the same in conventional and transition scenarios.

From an economic perspective, the price of chemical fertilisers, such as ammonium nitrate and superphosphates, increases steeply in 2021 but decreased again thereafter. Currently, the cost of ammonium nitrate is approximately 0.7 € kg⁻¹, considering that it is used on wheat at 100 kg ha⁻¹, this results in a cost of N fertilisation of approximately 70 € ha⁻¹. The price of triple-phosphate is approximately 0.4 € kg⁻¹, as is added at 200 kg ha⁻¹ (Falcone et al., 2019), resulting in 80€ ha⁻¹. Several pesticides are used on wheat, one of the most common is 2,4-Dichlorophenoxyacetic acid, which costs approximately 10 € L⁻¹ and is used at 0.5–2 L ha⁻¹ (Magnoli et al., 2020), resulting in a cost of 5–20 € ha⁻¹. In summary, the fertiliser and pesticide total expenditure per ha in the conventional system is approximately 160 € ha⁻¹. Micosat F1 has a global market value of 40 Mg year⁻¹. Currently, these microbial-based fertilisers (their cost is approximately 30€ kg⁻¹), when used at 10 kg ha⁻¹, cost 300€ ha⁻¹, thus they cannot compete with previous estimates, although their prices are predicted to drop within the next few years (Tensi et al., 2023). The price of biochar ranges from 150 to 300 € Mg⁻¹ (Haelderms et al., 2020; Struhs et al., 2020); therefore, if added at 2.5 Mg ha⁻¹, it costs 300–400 € ha⁻¹ which must be added to the expenditure for microbial fertilisers. The production of biochar sequesters carbon and this benefit can be monetised considering that the price of carbon has been estimated at 10–40 € Mg⁻¹ CO₂ (Campion et al., 2023). This advantage would be even more interesting if biochar was produced directly from the straw left after harvesting. Moreover, while fertilisers must be applied every year, the biochar remaining in the soil lasts for many years and undergoes only very slow weathering (Nepal et al., 2023; Williams et al., 2019). Overall, from an economic perspective, using biochar and PGPMs is still not competitive with the use of conventional fertilisers (the total cost has been estimated to be 5–6 fold higher than that of conventional fertilisation), even though there are important environmental aspects to be considered, in addition to CF savings.

Indeed, lowering, or eliminating N-P-K during fertilisation and maintaining organic fertilisers will reduce the risk of water

eutrophication, maintain an environment that supports aquatic life, and reduce N₂O emissions. It has been estimated that fertilisers cause eutrophication of 0.5 kg N-eq (Hasler et al., 2015), per 100 kg of fertiliser used. Water eutrophication can pollute drinking water supplies and enhance the growth of aquatic vegetation, such as blue-green algae, which in turn lowers sunlight penetration, thus decreasing the efficiency of photosynthesis in aquatic plants, inducing hypoxia in the water, and adversely affecting aquatic life (Akinawo, 2023). In addition, it is estimated that there is an emission of 1 kg of GHG N₂O in the atmosphere (Shcherbak et al., 2014; Stehfest and Bouwman, 2006) for each 100 kg of fertiliser used. An important point is the contribution of wheat cultivation and its category of impact which concerns not only the environment (acidification, and eutrophication of fresh and marine waters) but also human health (human toxicity and cancer effects, photochemical ozone formation, and resource depletion) (Falcone et al., 2019).

4. Conclusions

Microbial-based biofertilisers combined with amendments, such as biochar, can be a more manageable and sustainable alternative to classical fertilisation and support the European Green Deal, the “No waste” directive, and the “Farm to Fork” strategy while promoting circularity. All observed effects on the yield and quality of the wheat grains and flour and the composition of its protein fractions were correlated with the maintenance of a large microbial biodiversity in the soil, even though the climate recorded during the growing season and the filling period exerted a strong influence on the measured traits.

To date, the economic, political, and strategic roles of food production have been above any other consideration, and it has been reported, after a survey among EU farmers, that for some crops, these solutions are still not mature enough, at least in the hands of those (the farmers) who should benefit from them (Tensi et al., 2023). However, in addition to any economic considerations, it is also relevant that the continuous reduction in water resources and increase in pollutants, along with the new regulations on GHG emissions, will become a new driving force of economic value in a short time. These, combined with the public request for healthier food, are enough stimuli to continue the scientific dialogue with the production forces in a more global and holistic way.

CRedit authorship contribution statement

Marina Caldara: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Mariolina Gulli:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **Sara Graziano:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Nicolò Riboni:** Writing – review & editing. **Elena Maestri:** Writing – review & editing, Funding acquisition. **Monica Mattarozzi:** Writing – review & editing. **Federica Bianchi:** Writing – review & editing. **Maria Careri:** Writing – review & editing. **Nelson Marmiroli:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

The data presented in this study are openly available at NCBI database, BioProject under accession number PRJNA936795 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA936795>).

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Appendix A. Supplementary data

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References

- Akinawo, S.O., 2023. Eutrophication: causes, consequences, physical, chemical and biological techniques for mitigation strategies. *Environ. Challenges*. <https://doi.org/10.1016/j.envc.2023.100733>.
- Altenbach, S.B., Kothari, K.M., Lieu, D., 2002. Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. *Cereal Chem.* 79, 279–285. <https://doi.org/10.1094/CCHEM.2002.79.2.279>.
- Assainar, S.K., Abbott, L.K., Mickan, B.S., Whiteley, A.S., Siddique, K.H.M., Solaiman, Z.M., 2018. Response of wheat to a multiple species microbial inoculant compared to fertilizer application. *Front. Plant Sci.* 871 <https://doi.org/10.3389/fpls.2018.01601>.
- Bailey-Serres, J., Parker, J.E., Ainsworth, E.A., Oldroyd, G.E.D., Schroeder, J.I., 2019. Genetic strategies for improving crop yields. *Nature*. <https://doi.org/10.1038/s41586-019-1679-0>.
- Bakar, N.A., Karsani, S.A., Alias, S.A., 2020. Fungal survival under temperature stress: a proteomic perspective. *PeerJ* 8. <https://doi.org/10.7717/peerj.10423>.
- Basciani, S., Camajani, E., Contini, S., Persichetti, A., Risi, R., Bertoldi, L., Strigari, L., Prossomariti, G., Watanabe, M., Mariani, S., Lubrano, C., Genco, A., Spera, G., Gnassi, L., 2020. Very-low-calorie ketogenic diets with whey, vegetable, or animal protein in patients with obesity: a randomized pilot study. *J. Clin. Endocrinol. Metab.* 105, 2939–2949. <https://doi.org/10.1210/clinem/dgaa336>.
- Boukhater, Z.F., Merabet, C., Tsaki, H., 2022. Plant growth promoting actinobacteria, the most promising candidates as bioinoculants? *Front. Agron.* <https://doi.org/10.3389/fagro.2022.849911>.
- Bouskill, N.J., Wood, T.E., Baran, R., Hao, Z., Ye, Z., Bowen, B.P., Lim, H.C., Nico, P.S., Holman, H.Y., Gilbert, B., Silver, W.L., Northen, T.R., Brodie, E.L., 2016. Belowground response to drought in a tropical forest soil. II. Change in microbial function impacts carbon composition. *Front. Microbiol.* 7 <https://doi.org/10.3389/fmicb.2016.00323>.
- Brassard, P., Godbout, S., Pelletier, F., Raghavan, V., Palacios, J.H., 2018. Pyrolysis of switchgrass in an auger reactor for biochar production: a greenhouse gas and energy impacts assessment. *Biomass Bioenergy* 116, 99–105. <https://doi.org/10.1016/j.biombioe.2018.06.007>.
- Campion, L., Bekchanova, M., Malina, R., Kuppens, T., 2023. The costs and benefits of biochar production and use: a systematic review. *J. Clean. Prod.* <https://doi.org/10.1016/j.jclepro.2023.137138>.
- Cao, S., Xu, D., Hanif, M., Xia, X., He, Z., 2020. Genetic architecture underpinning yield component traits in wheat. *Theor. Appl. Genet.* <https://doi.org/10.1007/s00122-020-03562-8>.
- Dang, P., Vu, N.H., Shen, Z., Liu, J., Zhao, F., Zhu, H., Yu, X., Zhao, Z., 2018. Changes in soil fungal communities and vegetation following afforestation with *Pinus tabulaeformis* on the Loess Plateau. *Ecosphere* 9. <https://doi.org/10.1002/ecs2.2401>.
- Daniel, C., Triboi, E., 2001. Isolation of wheat grain compartments and their protein composition. *Cereal Res. Commun.* 29, 197–204. <https://doi.org/10.1007/bf03543661>.
- De la Rosa, L., Alvarez-Parrilla, E., Gonzalez-Aguilar, G.A., 2009. Chapter: synthesis and metabolism of phenolic compounds. In: *Fruit and Vegetable Phytochemicals, Fruit and Vegetable Phytochemicals*. <https://doi.org/10.1002/9780813809397>.
- De Santis, M.A., Kosik, O., Passmore, D., Flagella, Z., Shewry, P.R., Lovegrove, A., 2018. Comparison of the dietary fibre composition of old and modern durum wheat (*Triticum turgidum* spp. durum) genotypes. *Food Chem.* 244, 304–310. <https://doi.org/10.1016/j.foodchem.2017.09.143>.
- Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. A review. *Agron. Sustain. Dev.* <https://doi.org/10.1007/s13593-016-0372-z>.

- Du, J.X., Li, Y., Ur-Rehman, S., Mukhtar, I., Yin, Z., Dong, H., Wang, H., Zhang, X., Gao, Z., Zhao, X., Xin, X., Ding, X., 2021. Synergistically promoting plant health by harnessing synthetic microbial communities and prebiotics. *iScience*. <https://doi.org/10.1016/j.isci.2021.102918>.
- EL Sabagh, A., Islam, M.S., Skalicky, M., Ali Raza, M., Singh, K., Anwar Hossain, M., Hossain, A., Mahboob, W., Iqbal, M.A., Ratnasekera, D., Singhal, R.K., Ahmed, S., Kumari, A., Wasaya, A., Sytar, O., Brestic, M., ČIG, F., Erman, M., Habib Ur Rahman, M., Ullah, N., Arshad, A., 2021. Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: adaptation and management strategies. *Front. Agron.* <https://doi.org/10.3389/fagro.2021.661932>.
- Falcone, G., Stillitano, T., Montemurro, F., De Luca, A.I., Gulisano, G., Strano, A., 2019. Environmental and economic assessment of sustainability in mediterranean wheat production. *Agron. Res.* 17, 60–76. <https://doi.org/10.15159/AR.19.011>.
- FAO, 2021. The share of agri-food systems in total greenhouse gas emissions Global, regional and country trends. FAOSTAT Analytical Br. 31, 12.
- Farahani, H.A., Moaveni, P., Maroufi, K., 2011. Effect of seed size on seedling production in wheat (*Triticum aestivum* L.). *Adv. Environ. Biol.* 5, 1711–1715.
- Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/nrmicro.2017.87>.
- Fiorilli, V., Maghrebi, M., Novero, M., Votta, C., Mazzarella, T., Buffoni, B., Astolfi, S., Vigani, G., 2022. Arbuscular mycorrhizal symbiosis differentially affects the nutritional status of two durum wheat genotypes under drought conditions. *Plants* 11. <https://doi.org/10.3390/plants11060804>.
- Flagella, Z., Giuliani, M.M., Giuzio, L., Volpi, C., Masci, S., 2010. Influence of water deficit on durum wheat storage protein composition and technological quality. *Eur. J. Agron.* 33, 197–207. <https://doi.org/10.1016/j.eja.2010.05.006>.
- Gale, K.R., 2005. Diagnostic DNA markers for quality traits in wheat. *J. Cereal Sci.* 41, 181–192. <https://doi.org/10.1016/j.jcs.2004.09.002>.
- Gan, Y., Liang, C., Wang, X., McConkey, B., 2011. Lowering carbon footprint of durum wheat by diversifying cropping systems. *F. Crop Res.* 122, 199–206.
- García-Molina, M.D., Barro, F., 2017. Characterization of changes in gluten proteins in low-gliadin transgenic wheat lines in response to application of different nitrogen regimes. *Front. Plant Sci.* 8 <https://doi.org/10.3389/fpls.2017.00257>.
- Graziano, S., Marando, S., Prandi, B., Boukid, F., Marmiroli, N., Francia, E., Pecchioni, N., Sforza, S., Visioli, G., Gulli, M., 2019. Technological quality and nutritional value of two durum wheat varieties depend on both genetic and environmental factors. *J. Agric. Food Chem.* 67, 2384–2395. <https://doi.org/10.1021/acs.jafc.8b06621>.
- Graziano, S., Marmiroli, N., Visioli, G., Gulli, M., 2020. Proteins and metabolites as indicators of flours quality and nutritional properties of two durum wheat varieties grown in different Italian locations. *Foods* 9. <https://doi.org/10.3390/foods9030315>.
- Graziano, S., Caldara, M., Gulli, M., Bevivino, A., Maestri, E., Marmiroli, N., 2022. A metagenomic and gene expression analysis in wheat (*T. Durum*) and maize (*Z. mays*) biofertilized with PGPM and biochar. *Int. J. Mol. Sci.* <https://doi.org/10.3390/ijms231810376>.
- Gruber, K., 2015. Deep influence of soil microbes. *Nat. Plants.* <https://doi.org/10.1038/NPLANTS.2015.194>.
- Gujre, N., Soni, A., Rangan, L., Tsang, D.C.W., Mitra, S., 2021. Sustainable improvement of soil health utilizing biochar and arbuscular mycorrhizal fungi: a review. *Environ. Pollut.* <https://doi.org/10.1016/j.envpol.2020.115549>.
- Gulli, M., De Pascali, M., Perrotta, C., Rampino, P., 2022. A stress-related transcription factor belonging to the YL-1 family is differently regulated in durum wheat cultivars differing in drought sensitivity. *Plant Physiol. Biochem.* 170, 307–315. <https://doi.org/10.1016/j.plaphy.2021.12.016>.
- Guzmán, C., Posadas-Romano, G., Hernández-Espinosa, N., Morales-Dorantes, A., Peña, R.J., 2015. A new standard water absorption criteria based on solvent retention capacity (SRC) to determine dough mixing properties, viscoelasticity, and bread-making quality. *J. Cereal Sci.* 66, 59–65. <https://doi.org/10.1016/j.jcs.2015.10.009>.
- Haeldermans, T., Campion, L., Kuppens, T., Vanreppelen, K., Cuypers, A., Schreurs, S., 2020. A comparative techno-economic assessment of biochar production from different residue streams using conventional and microwave pyrolysis. *Bioresour. Technol.* 318 <https://doi.org/10.1016/j.biortech.2020.124083>.
- Hamedani, S.R., Kuppens, T., Malina, R., Bocci, E., Colantoni, A., Villarini, M., 2019. Life cycle assessment and environmental valuation of biochar production: two case studies in Belgium. *Energies* 12. <https://doi.org/10.3390/en12112166>.
- Hasler, K., Bröring, S., Omta, S.W.F., Olfs, H.W., 2015. Life cycle assessment (LCA) of different fertilizer product types. *Eur. J. Agron.* 69, 41–51. <https://doi.org/10.1016/j.eja.2015.06.001>.
- He, M., Xiong, X., Wang, L., Hou, D., Bolan, N.S., Ok, Y.S., Rinklebe, J., Tsang, D.C.W., 2021a. A critical review on performance indicators for evaluating soil biota and soil health of biochar-amended soils. *J. Hazard. Mater.* 414 <https://doi.org/10.1016/j.jhazmat.2021.125378>.
- He, M., Xu, Z., Sun, Y., Chan, P.S., Lui, I., Tsang, D.C.W., 2021b. Critical impacts of pyrolysis conditions and activation methods on application-oriented production of wood waste-derived biochar. *Bioresour. Technol.* 341 <https://doi.org/10.1016/j.biortech.2021.125811>.
- HGCA, 2012. Understanding carbon footprinting for cereals and oilseeds [WWW Document]. www.hgca.com.
- Hou, L., Yang, Y., Zhang, X., Jiang, C., 2021. Carbon footprint for wheat and maize production modulated by farm size: a study in the North China plain. *Int. J. Clim. Chang. Strateg. Manag.* 13, 302–319. <https://doi.org/10.1108/IJCCSM-10-2020-0110>.
- Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunt, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I.C., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S. A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., De Hoog, S., Kang, J. C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A. N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yacharoen, S., Yan, J.Y., Zhang, M., 2013. Families of dothideomycetes. *Fungal Divers.* 63, 1–313. <https://doi.org/10.1007/s13225-013-0263-4>.
- Igalavithana, A.D., Ok, Y.S., Usman, A.R.A., Al-Wabel, M.I., Oleszczuk, P., Lee, S.S., 2015. The Effects of Biochar Amendment on Soil Fertility. SSSA Special Publications. <https://doi.org/10.2136/sssaspecpub63.2014.0040>.
- Igrejas, G., Ikeda, T.M., Guzmán, C., 2020. Wheat quality for improving processing and human health. In: *Wheat Quality For Improving Processing And Human Health*. <https://doi.org/10.1007/978-3-030-34163-3>.
- International Organization for Standardization (ISO), 2016. EN ISO 17294-2:2016 Water Quality. Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Determination of Selected Elements Including Uranium Isotopes.
- Janni, M., Gulli, M., Maestri, E., Marmiroli, M., Valliyodan, B., Nguyen, H.T., Marmiroli, N., Foyer, C., 2020. Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. *J. Exp. Bot.* <https://doi.org/10.1093/jxb/eraa034>.
- Ji, G., Xu, Z., Fan, X., Zhou, Q., Chen, L., Yu, Q., Liao, S., Jiang, C., Feng, B., Wang, T., 2023. Identification and validation of major QTL for grain size and weight in bread wheat (*Triticum aestivum* L.). *Crop J.* 11, 564–572. <https://doi.org/10.1016/cj.2022.06.014>.
- Juhmani, A.S., Vezzi, A., Wahsha, M., Buosi, A., De Pascale, F., Schiavon, R., Sfriso, A., 2020. Diversity and dynamics of seaweed associated microbial communities inhabiting the lagoon of Venice. *Microorganisms* 8, 1–23. <https://doi.org/10.3390/microorganisms8111657>.
- Kalam, S., Basu, A., Ahmad, I., Sayyed, R.Z., El-Enshasy, H.A., Dailin, D.J., Suriani, N.L., 2020. Recent understanding of soil acidobacteria and their ecological significance: a critical review. *Front. Microbiol.* <https://doi.org/10.3389/fmicb.2020.580024>.
- Kashyap, D., Agarwal, T., 2021. Carbon footprint and water footprint of rice and wheat production in Punjab, India. *Agric. Syst.* 186 <https://doi.org/10.1016/j.agsy.2020.102959>.
- Kumar, S., Diksha, Sindhu, S.S., Kumar, R., 2022. Biofertilizers: an ecofriendly technology for nutrient recycling and environmental sustainability. *Curr. Res. Microb. Sci.* <https://doi.org/10.1016/j.crmicr.2021.100094>.
- Li, J., Liu, X., Yang, X., Li, Y., Wang, C., He, D., 2018. Proteomic analysis of the impacts of powdery mildew on wheat grain. *Food Chem.* 261, 30–35. <https://doi.org/10.1016/j.foodchem.2018.04.024>.
- Li, C., Xu, M., Cai, X., Han, Z., Si, J., Chen, D., 2022. Jasmonate signaling pathway modulates plant defense, growth, and their trade-offs. *Int. J. Mol. Sci.* <https://doi.org/10.3390/ijms23073945>.
- Liu, H., Zhang, X., Xu, Y., Ma, F., Zhang, J., Cao, Y., Li, L., An, D., 2020. Identification and validation of quantitative trait loci for kernel traits in common wheat (*Triticum aestivum* L.). *BMC Plant Biol.* 20 <https://doi.org/10.1186/s12870-020-02661-4>.
- Long, S.P., Zhu, X.G., Naidu, S.L., Ort, D.R., 2006. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* <https://doi.org/10.1111/j.1365-3040.2005.01493.x>.
- Lopes, M.J., Dos S., Dias-Filho, M.B., Gurgel, E.S.C., 2021. Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Front. Sustain. Food Syst.* <https://doi.org/10.3389/fsufs.2021.606454>.
- Lupini, A., Preiti, G., Badagliacca, G., Abenavoli, M.R., Sunseri, F., Monti, M., Bacchi, M., 2021. Nitrogen use efficiency in durum wheat under different nitrogen and water regimes in the Mediterranean Basin. *Front. Plant Sci.* 11 <https://doi.org/10.3389/fpls.2020.607226>.
- Magnoli, K., Carranza, C.S., Aluffi, M.E., Magnoli, C.E., Barberis, C.L., 2020. Herbicides based on 2,4-D: its behavior in agricultural environments and microbial biodegradation aspects. A review. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-020-10370-6>.
- Mäkinen, H., Kaseva, J., Trnka, M., Balek, J., Kersebaum, K.C., Nendel, C., Gobin, A., Olesen, J.E., Bindi, M., Ferrise, R., Moriondo, M., Rodríguez, A., Ruiz-Ramos, M., Takáč, J., Bezák, P., Ventrella, D., Ruget, F., Capellades, G., Kahiluoto, H., 2018. Sensitivity of European wheat to extreme weather. *F. Crop Res.* 222, 209–217. <https://doi.org/10.1016/j.fcr.2017.11.008>.
- Makino, A., 2011. Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. *Plant Physiol.* 155, 125–129. <https://doi.org/10.1104/pp.110.165076>.
- Manfredi, A., Mattarozzi, M., Giannetto, M., Careri, M., 2015. Multiplex liquid chromatography-tandem mass spectrometry for the detection of wheat, oat, barley and rye prolamins towards the assessment of gluten-free product safety. *Anal. Chim. Acta* 895, 62–70. <https://doi.org/10.1016/j.aca.2015.09.008>.
- Marčiulyrienė, D., Marčiulynas, A., Mishcherikova, V., Lynikienė, J., Gedminas, A., Franc, I., Menkis, A., 2022. Principal drivers of fungal communities associated with needles, shoots, roots and adjacent soil of *Pinus sylvestris*. *J. Fungi* 8. <https://doi.org/10.3390/jof8101112>.
- Marmiroli, M., Bonas, U., Imperiale, D., Lencioni, G., Mussi, F., Marmiroli, N., Maestri, E., 2018. Structural and functional features of chars from different biomasses as potential plant amendments. *Front. Plant Sci.* 9 <https://doi.org/10.3389/fpls.2018.01119>.
- Marmiroli, M., Caldara, M., Pantalone, S., Malcevski, A., Maestri, E., Marmiroli, N., 2022. Building a risk matrix for the safety assessment of wood derived biochars. *Sci. Total Environ.* 15, 156265.

- Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D.D., 1998. Characterization of a low-molecular-weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. *Plant Physiol.* 118, 1147–1158. <https://doi.org/10.1104/pp.118.4.1147>.
- Min, B., Salt, L., Wilde, P., Kosik, O., Hassall, K., Przewieslik-Allen, A., Burrige, A.J., Poole, M., Snape, J., Wingen, L., Haslam, R., Griffiths, S., Shewry, P.R., 2020. Genetic variation in wheat grain quality is associated with differences in the galactolipid content of flour and the gas bubble properties of dough liquor. *Food Chem. X* 6. <https://doi.org/10.1016/j.foodchem.2020.100093>.
- Ministro per le Politiche Agricole, 1999. Metodi ufficiali di analisi chimica del suolo, DM 13/09/1999 SO n° 185 GU n° 248 21/10/1999.
- Mohammadi-Sichani, M.M., Assadi, M.M., Farazmand, A., Kianirad, M., Ahadi, A.M., Ghahderjani, H.H., 2017. Bioremediation of soil contaminated crude oil by Agaricomycetes. *J. Environ. Heal. Sci. Eng.* 15 <https://doi.org/10.1186/s40201-016-0263-x>.
- Moshawh, S., Abdullah Juperi, R.N.A., Paneerselvam, G.S., Ming, L.C., Liew, K. Bin, Goh, B.H., Al-Worafi, Y.M., Choo, C.Y., Thuraisingam, S., Goh, H.P., Kifli, N., 2022. General health benefits and pharmacological activities of *Triticum aestivum* L. *Molecules*. <https://doi.org/10.3390/molecules27061948>.
- Naik, K., Mishra, S., Srichandan, H., Singh, P.K., Sarangi, P.K., 2019. Plant growth promoting microbes: potential link to sustainable agriculture and environment. *Biocatal. Agric. Biotechnol.* <https://doi.org/10.1016/j.bcab.2019.101326>.
- Naylor, D., Degraaf, S., Purdom, E., Coleman-Derr, D., 2017. Drought and host selection influence bacterial community dynamics in the grass root microbiome. *ISME J.* 11, 2691–2704. <https://doi.org/10.1038/ismej.2017.118>.
- Nepal, J., Ahmad, W., Munsif, F., Khan, A., Zou, Z., 2023. Advances and prospects of biochar in improving soil fertility, biochemical quality, and environmental applications. *Front. Environ. Sci.* <https://doi.org/10.3389/fenvs.2023.1114752>.
- Noulas, C., Tziouvalakas, M., Karyotis, T., 2018. Zinc in soils, water and food crops. *J. Trace Elem. Med. Biol.* <https://doi.org/10.1016/j.jtemb.2018.02.009>.
- Olckers, S.L.E., Osthoff, G., Guzmán, C., Wentzel, B., van Bijljon, A., Labuschagne, M., 2022. Drought and heat stress effects on gluten protein composition and its relation to bread-making quality in wheat. *J. Cereal Sci.* 108 <https://doi.org/10.1016/j.jcs.2022.103562>.
- Phakela, K., van Bijljon, A., Wentzel, B., Guzman, C., Labuschagne, M.T., 2021. Gluten protein response to heat and drought stress in durum wheat as measured by reverse phase - high performance liquid chromatography. *J. Cereal Sci.* 100 <https://doi.org/10.1016/j.jcs.2021.103267>.
- Pichereaux, C., Laurent, E.A., Gargasos, A., Viudes, S., Durieu, C., Lamaze, T., Grieu, P., Burel-Schiltz, O., 2019. Analysis of durum wheat proteome changes under marine and fungal biostimulant treatments using large-scale quantitative proteomics: a useful dataset of durum wheat proteins. *J. Proteomics* 200, 28–39. <https://doi.org/10.1016/j.jprot.2019.03.003>.
- Pratim, C., Sukanta, M., Shohini, C., Mamun, M., 2022. Rhizosphere Engineering, Chapter 12 - Zinc Solubilizing Rhizobacteria as Soil Health Engineer Managing Zinc Deficiency in Plants.
- Rashmi, I., Roy, T., Kartika, K.S., Pal, R., Coumar, V., Kala, S., Shinoji, K.C., 2020. Organic and inorganic fertilizer contaminants in agriculture: impact on soil and water resources. In: *Contaminants in Agriculture: Sources, Impacts and Management*, pp. 3–41. https://doi.org/10.1007/978-3-030-41552-5_1.
- Regione Emilia-Romagna, 2021. Il sistema agro-alimentare dell'Emilia-Romagna. Rapporto 2020.
- Regione Emilia-Romagna, 2022. Il sistema agro-alimentare dell'Emilia-Romagna. Rapporto 2021.
- 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. *J. Plant Nutr. Soil Sci.* 183, 260–270. <https://doi.org/10.1002/jpln.201900529>.
- Riboni, N., Bianchi, F., Mattarozzi, M., Caldara, M., Gulli, M., Graziano, S., Maestri, E., Marmiroli, N., Careri, M., 2023. Ultra-high performance liquid chromatography-ion mobility-high-resolution mass spectrometry to evaluate the metabolomic response of durum wheat to sustainable treatments. *J. Agric. Food Chem.* <https://doi.org/10.1021/acs.jafc.3c04532>.
- Ronga, D., Laviano, L., Catellani, M., Milc, J., Prandi, B., Boukid, F., Sforza, S., Dossena, A., Graziano, S., Gulli, M., Visioli, G., Marmiroli, N., De Vita, P., Pecchioni, N., Francia, E., 2020. Influence of environmental and genetic factors on content of toxic and immunogenic wheat gluten peptides. *Eur. J. Agron.* 118 <https://doi.org/10.1016/j.eja.2020.126091>.
- Ruiz-Dueñas, F.J., Barrasa, J.M., Sánchez-García, M., Camarero, S., Miyachi, S., Serrano, A., Linde, D., Babiker, R., Drula, E., Ayuso-Fernández, I., Pacheco, R., Padilla, G., Ferreira, P., Barriuso, J., Kellner, H., Castanera, R., Alfaro, M., Ramírez, L., Pisabarro, A.G., Riley, R., Kuo, A., Andreopoulos, W., Labutti, K., Pangilinan, J., Tritt, A., Lipzen, A., He, G., Yan, M., Ng, V., Grigoriev, I.V., Cullen, D., Martin, F., Rosso, M.N., Henrissat, B., Hibbett, D., Martínez, A.T., 2021. Genomic analysis enlightens Agaricales lifestyle evolution and increasing peroxidase diversity. *Mol. Biol. Evol.* 38, 1428–1446. <https://doi.org/10.1093/molbev/msaa301>.
- Sadeghi-Tehrani, P., Sabermanesh, K., Virel, N., Hawkesford, M.J., 2017. Automated method to determine two critical growth stages of wheat: heading and flowering. *Front. Plant Sci.* 8 <https://doi.org/10.3389/fpls.2017.00252>.
- Sansinenea, E., 2019. *Bacillus* spp.: as plant growth-promoting bacteria. In: *Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms: Discovery and Applications*, pp. 225–237. https://doi.org/10.1007/978-981-13-5862-3_11.
- Savci, S., 2012a. An agricultural pollutant: chemical fertilizer. *Int. J. Environ. Sci. Dev.* 73–80 <https://doi.org/10.7763/ijesd.2012.v3.191>.
- Savci, S., 2012b. Investigation of effect of chemical fertilizers on environment. *APCBEE Proc.* 1, 287–292. <https://doi.org/10.1016/j.apcb.2012.03.047>.
- Shcherbak, I., Millar, N., Robertson, G.P., 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N₂O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9199–9204. <https://doi.org/10.1073/pnas.1322434111>.
- Shen, N., Wang, T., Gan, Q., Wang, L., Jin, B., 2022. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. *Food Chem.* <https://doi.org/10.1016/j.foodchem.2022.132531>.
- Shepherd, S., 2014. Short-chain fermentable carbohydrates. In: *Advanced Nutrition and Dietetics in Gastroenterology*, pp. 72–80. <https://doi.org/10.1002/9781118872796.ch2.2>.
- Shevchenko, A., Tomas, H., Havliš, J., Olsen, J.V., Mann, M., 2007. In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat. Protoc.* 1, 2856–2860. <https://doi.org/10.1038/nprot.2006.468>.
- Shewry, P.R., Hey, S.J., 2015. The contribution of wheat to human diet and health. *Food Energy Secur.* <https://doi.org/10.1002/FES3.64>.
- Shiwakoti, S., Zheljzkov, V.D., Gollany, H.T., King, B., Kleber, M., 2019. Micronutrient concentrations in soil and wheat decline by long-term tillage and winter wheat-pear rotation. *Agronomy* 9. <https://doi.org/10.3390/agronomy9070359>.
- Shiwakoti, S., Zheljzkov, V.D., Gollany, H.T., Kleber, M., King, B., Astatkie, T., 2020. Macronutrient in soils and wheat from long-term agroexperiments reflects variations in residue and fertilizer inputs. *Sci. Rep.* 10 <https://doi.org/10.1038/s41598-020-60164-6>.
- Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. *ISME J.* 3, 992–1000. <https://doi.org/10.1038/ismej.2009.43>.
- Spani, R.C., 2020. The New Circular Economy Action Plan. SSRN.
- Stehfest, E., Bouwman, L., 2006. N₂O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutr. Cycl. Agroecosyst.* 74, 207–228. <https://doi.org/10.1007/s10705-006-9000-7>.
- Struhs, E., Mirkouei, A., You, Y., Mohajeri, A., 2020. Techno-economic and environmental assessments for nutrient-rich biochar production from cattle manure: a case study in Idaho, USA. *Appl. Energy* 279. <https://doi.org/10.1016/j.apenergy.2020.115782>.
- Sun, Y., Xiong, X., He, M., Xu, Z., Hou, D., Zhang, W., Ok, Y.S., Rinkebe, J., Wang, L., Tsang, D.C.W., 2021. Roles of biochar-derived dissolved organic matter in soil amendment and environmental remediation: a critical review. *Chem. Eng. J.* <https://doi.org/10.1016/j.cej.2021.130387>.
- Tabacchioni, S., Passato, S., Ambrosino, P., Huang, L., Caldara, M., Cantale, C., Hett, J., Del Fiore, A., Fiore, A., Schlüter, A., Sczyrba, A., Maestri, E., Marmiroli, N., Neuhoof, D., Nesme, J., Sorensen, S.J., Aprea, G., Nobili, C., Presenti, O., Giovannetti, G., Giovannetti, C., Pihlanto, A., Brunori, A., Bevilino, A., 2021. Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for a sustainable agriculture. *Microorganisms* 9, 1–23. <https://doi.org/10.3390/microorganisms9020426>.
- Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One*. <https://doi.org/10.1371/journal.pone.0105592>.
- Tensi, A.F., Ang, F., van der Fels-Klerx, H.J., 2023. Microbial applications and agricultural sustainability: a simulation analysis of Dutch potato farms. *SSRN Electron. J.* <https://doi.org/10.2139/ssrn.4357580>.
- Tshering, K., Rengel, Z., Storer, P., Solaiman, Z.M., 2022. Microbial consortium inoculum with rock minerals increased wheat grain yield, nitrogen-use efficiency, and protein yield due to larger root growth and architecture. *Agronomy* 12. <https://doi.org/10.3390/agronomy12102481>.
- Tubiello, F.N., Flammini, A., Karl, K., Obli-Laryea, G., Qiu, S.Y., Heiðarsdóttir, H., Pan, X., Conchedda, G., 2021. Methods for estimating greenhouse gas emissions from food systems. Part III: energy use in fertilizer manufacturing, food processing, packaging, retail and household consumption. *FAO Stat. Work. Pap. Ser.* 29 <https://doi.org/10.4060/cb7473en>.
- UNI.EN, 2012. n.d. 16174 Sludge, Treated Biowaste and soil - Digestion of aqua regia Soluble Fractions of Elements.
- van Bergeijk, D.A., Terlouw, B.R., Medema, M.H., van Wezel, G.P., 2020. Ecology and genomics of Actinobacteria: new concepts for natural product discovery. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-020-0379-y>.
- Visioli, G., Bonas, U., Dal Cortivo, C., Pasini, G., Marmiroli, N., Mosca, G., Vamerli, T., 2018. Variations in yield and gluten proteins in durum wheat varieties under late-season foliar versus soil application of nitrogen fertilizer in a northern Mediterranean environment. *J. Sci. Food Agric.* 98, 2360–2369. <https://doi.org/10.1002/jsfa.8727>.
- Vocciante, M., Griffoni, M., Fusini, D., Petruzzelli, G., Franchi, E., 2022. The role of plant growth-promoting Rhizobacteria (PGPR) in mitigating plant's environmental stresses. *Appl. Sci.* <https://doi.org/10.3390/app12031231>.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols*. <https://doi.org/10.1016/b978-0-12-372180-8.50042-1>.
- Wieser, H., Koehler, P., Scherf, K.A., 2020a. Chemical composition of wheat grains. In: *Wheat-An Exceptional Crop*, pp. 13–45. <https://doi.org/10.1016/b978-0-12-821715-3.00002-2>.
- Wieser, H., Koehler, P., Scherf, K.A., 2020b. The two faces of wheat. *Front. Nutr.* <https://doi.org/10.3389/fnut.2020.517313>.
- Williams, E.K., Jones, D.L., Sanders, H.R., Benitez, G.V., Plante, A.F., 2019. Effects of 7 years of field weathering on biochar recalcitrance and solubility. *Biochar* 1, 237–248. <https://doi.org/10.1007/s42773-019-00026-1>.
- Wolińska, A., Kuźniar, A., Zielenkiewicz, U., Izak, D., Szafranek-Nakonieczna, A., Banach, A., Błaszczak, M., 2017. Bacteroidetes as a sensitive biological indicator of

- agricultural soil usage revealed by a culture-independent approach. *Appl. Soil Ecol.* 119, 128–137. <https://doi.org/10.1016/j.apsoil.2017.06.009>.
- Würschum, T., Langer, S.M., Longin, C.F.H., 2015. Genetic control of plant height in European winter wheat cultivars. *Theor. Appl. Genet.* 128, 865–874. <https://doi.org/10.1007/s00122-015-2476-2>.
- Yadav, R., Ror, P., Beniwal, R., Kumar, S., Ramakrishna, W., 2022. *Bacillus* sp. and arbuscular mycorrhizal fungi consortia enhance wheat nutrient and yield in the second-year field trial: superior performance in comparison with chemical fertilizers. *J. Appl. Microbiol.* 132, 2203–2219. <https://doi.org/10.1111/jam.15371>.
- Yang, J., Xie, B., Bai, J., Yang, Q., 2012. Purification and characterization of a nitroreductase from the soil bacterium *Streptomyces mirabilis*. *Process Biochem.* 47, 720–724. <https://doi.org/10.1016/j.procbio.2012.01.021>.
- Zhong, Y., Xu, D., Hebelstrup, K.H., Yang, D., Cai, J., Wang, X., Zhou, Q., Cao, W., Dai, T., Jiang, D., 2018. Nitrogen topdressing timing modifies free amino acids profiles and storage protein gene expression in wheat grain. *BMC Plant Biol.* 18 <https://doi.org/10.1186/s12870-018-1563-3>.
- Zingale, S., Spina, A., Ingraio, C., Fallico, B., Timpanaro, G., Anastasi, U., Guarnaccia, P., 2023. Factors affecting the nutritional, health, and technological quality of durum wheat for pasta-making: a systematic literature review. *Plants* 12, 530.