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# Combining wheat with multi-species prairie strips reduces the development of *Septoria tritici* blotch

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## ABSTRACT

Crop diversification, notably through intercropping, is known for regulating disease development in agroecosystems. However, intercropping usually involves two or three species, limiting its potential to enhance ecosystem multifunctionality. This study investigates the impact of “agroprairies”, a cropping system design consisting of alternating narrow strips of wheat and multispecies prairies (~12 species), on the development of *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch (STB), a major foliar disease in wheat. Two complementary field experiments were conducted. The first one monitored the incidence and severity of STB under natural epidemic conditions across combinations of four wheat cultivars and four prairie mixtures with contrasted functional traits. The second experiment used above-canopy inoculation on a subset of treatments to assess disease dynamics under high disease pressure, independent of physical barrier effects. Rhizosphere microbiomes were analyzed before inoculation to evaluate enrichment in taxa associated with plant defense and disease suppression. Under natural conditions, STB incidence and severity were reduced by 52% and 41% in two agroprairie types compared to monocrop. Prairie biomass and specific leaf area (SLA) were identified as key factors associated with this reduction. When the disease was inoculated above the canopy, this reduction effect disappeared, highlighting the dominant role of physical barriers in disease regulation. However, one agroprairie treatment still reduced disease progression by 18%, suggesting the involvement of additional regulatory mechanisms. Microbiome analysis revealed shifts in bacterial beta diversity and an enrichment of potentially beneficial taxa in the wheat rhizosphere, although no direct link with disease regulation was established.

## 1. Introduction

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici* (Sánchez-Vallet et al., 2015) is one of the most damaging leaf diseases of wheat (Suarez-Fernandez and De Francesco, 2024), the most widely grown small grain cereal in the world (Balfourier et al., 2019; Erenstein et al., 2022). The fungal pathogen disperses via wind in the acrospore (sexual) form from distant infected wheat debris and then via rain splash from the bottom to the top of the plant in the

pycnidiospores (asexual) form (Shaw, 1987; Steinberg, 2015; Suffert et al., 2011). The risk of contamination extends from the two-node stage to the flowering stage. After contamination, the development of STB causes progressive leaf necrosis, which can lead to yield losses of up to 20% in the absence of effective disease management strategies (Fones and Gurr, 2015). The most common methods of managing this disease are using resistant wheat cultivars or applying fungicides (Sánchez-Vallet et al., 2015). However, *Z. tritici* evolves rapidly, and can develop resistance to these practices (Jørgensen et al., 2022; McDonald

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and Mundt, 2016). Furthermore, concerns about the environmental and health impacts of pesticides have led to the European Green Deal that aims to reduce pesticide use by 50% by 2030 (Boix-Fayos and de Vente, 2023).

Crop diversification at the field scale is an effective way to limit disease development (Vialatte et al., 2025). Among the crop diversification methods, intercropping is one of the most effective approaches (Beillouin et al., 2021) and several studies showed a reduction of leaf diseases in intercropping compared to monocrops (De Long et al., 2023; Zhang et al., 2019). For example, Zhang et al., (2019) demonstrated that wheat-faba bean intercrops reduced yellow rust and powdery mildew by 34% and 20% respectively.

Yet, current intercropping systems typically involve two or three plant species. However, studies in grassland ecosystems have demonstrated that the maximum efficiency of reducing disease severity can be achieved with much richer plant communities (12 plant species in Mitchell et al., 2002). If we consider functions other than disease regulation, such as biomass production, pollination, biological control of pests and weeds, soil carbon storage, nutrient retention, as well as their resistance to climatic fluctuations, the level of diversification required to maximize ecosystem functions is likely even more important (Gross et al., 2017; Newberger et al., 2023; Wagg et al., 2014). To address the challenge of increasing plant diversity at the field scale and improving ecosystem functioning, including disease regulation, a novel cropping system called "agroprairie" was designed. In reference to agroforestry, agroprairies consist of a permanent, diversified grassland plant community that is arranged in narrow, alternating strips with annual crops as presented in our conceptual model (Fig. 1). To foster and test the coexistence of a large number of species in the prairie strips over years, we used different mixtures of species commonly found in natural or semi-natural prairies of the studied area, and differently positioned on two different plant functional dimensions. The first dimension relates to resource use strategy of plants, that distinguishes "resource-acquisitive" and "resource-conservative" plant species (Grime, 2006). Resource-acquisitive species are characterized by rapid growth, high tissue turnover, litter chemistry that promotes microbial decomposition, and high nutrient uptake from the soil (Henneron et al., 2020). In contrast, "resource-conservative" species are characterized by slow growth (Lambers and Poorter, 1992), low tissue turnover, litter chemistry resistant to microbial decomposition and low nutrient uptake from soil (Henneron et al., 2020; Lambers and Poorter, 1992). The second plant functional dimension also used to differentiate plant communities of the prairie strips was their ability to fix atmospheric N, i.e the proportion of legumes in the plant community.

The strip intercropping of wheat with multi-species prairie can play a role in regulating disease development through various possible mechanisms known from intercropping systems, as summarized in the conceptual model (Fig. 1). The first mechanism involved is the interference with spore dispersal by wind or rain in relation to barrier effect of

companion plants. This mechanism is often the first suggested in intercropping field studies (Luo et al., 2022; Zhang et al., 2019). Second, host morphology and physiology alteration in response to the intercropping practice can also modify disease development, modifying microclimate and resources for pathogen (Richard et al., 2013; Vidal et al., 2017). This includes, for example, changes in the host plant's architecture and the leaf nitrogen content (Gómez-Rodríguez et al., 2007; Wu et al., 2024). A third possible mechanism is the host sensitivity/defense modification partly linked with the recruitment of a specific microbiome in the host plant generated by intercropping (Mao et al., 2024; H. Yu et al., 2019; Zhu et al., 2024). A key site for microbiome-related changes appears to be the plant rhizosphere, where microbial communities are believed to control diseases by triggering defense responses or producing bioactive compounds (Berendsen et al., 2018; H. Liu et al., 2020). These microbial communities may act locally within the rhizosphere (H. Liu et al., 2020; Liu et al., 2023) or subsequently colonize other plant tissues (Dini-Andreote, 2020). However, the role of rhizosphere microbiome in regulating plant responses to pathogens is much less understood than physical or physiological aspects in the field, even if studies reported positive effects of diversification practices on microbial diversity (Granzow et al., 2017; Jin et al., 2020; Pang et al., 2022; H. Yu et al., 2019).

The main objective of this study is to determine whether the development of STB in wheat is influenced by the agroprairie design, i.e. the strip intercropping of wheat with multispecies prairie, and to investigate the potential mechanisms involved in the regulation of STB. To do so, we have combined two complementary experiments. The first study aimed to identify how changes in STB incidence and severity under natural epidemic conditions relate to the use of the agroprairie design and to specific prairie plant communities and wheat cultivars. Our hypotheses were that the contrasting biomasses, heights and leaf traits of the intercropped prairie strips would generate different barrier effect efficiencies (Fig. 1). Also, because wheat cultivars were chosen for their contrasting disease sensitivities and phenotype (e.g. maximum height), we expected different disease incidence and severity outcomes, depending on wheat cultivar response to intercropping situations.

The second experiment aimed to assess whether the wheat-prairie strip intercropping alter plant sensitivity and defense against STB, potentially through prairie-induced changes in the wheat rhizosphere microbiome. In this experiment, wheat plants were inoculated with *Z. tritici*, thereby eliminating the potential barrier effect of the prairie strip and allowed for a targeted evaluation of prairie influences on wheat sensitivity and defense responses. A focus was made on the most sensitive wheat cultivar and the two most contrasting prairie communities in terms of species composition and functional traits. The potential effect of intercropped prairies on wheat sensitivity and defense, as well as their link to shift in the wheat rhizosphere microbiome, were studied by characterizing the dynamics of STB on inoculated plants and analyzing the rhizosphere microbiomes of associated prairies and wheat (Fig. 1).

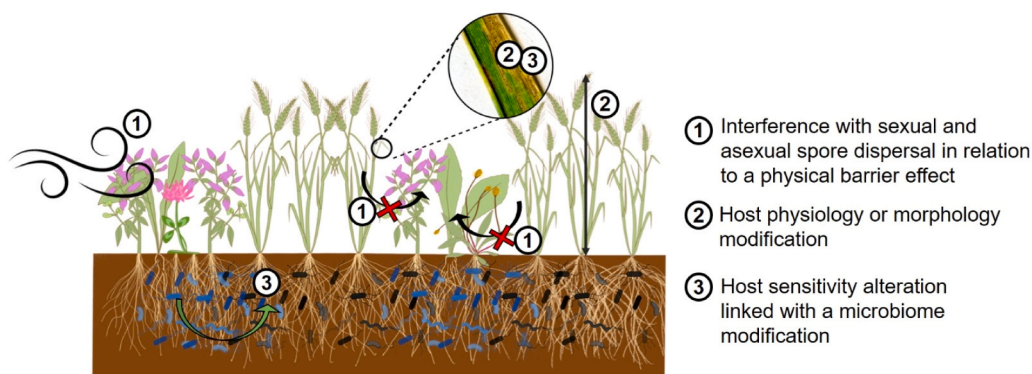


Fig. 1. Conceptual model of disease regulation mechanisms potentially involved in a wheat-prairie strip cropping system.

We deepened our analysis of the wheat rhizosphere microbiome by researching microbial taxa known to contribute to plant defense and pathogen suppression. We hypothesized that prairie species differing in resource-use strategies and functional traits would harbor distinct rhizosphere microbiomes, thereby shaping the wheat rhizosphere microbiome and wheat sensitivity to STB.

## 2. Material and methods

### 2.1. Site description

The two experiments were carried out in 2023–2024 at the INRAE Crouel Research Station, in Clermont-Ferrand in France (45°46'42.75"N, 3°8'48.79"E, elevation 331 m a.s.l.). The soil is a calcareous silty clay-loam soil. Soil characteristics are indicated in Table S.1. Mean annual temperatures was 14 and 13°C in 2023 and 2024 respectively. Mean annual precipitation was 518 and 736 mm in 2023 and 2024, respectively. Before the experiments, a maize was cultivated in 2022, a winter wheat in 2021 and a soybean in 2020.

### 2.2. Agroprairie design and field management

“Agroprairie” design consisted of micro-plots (3 m × 3.5 m) with four alternating strips of prairie and wheat, each 37.5 cm wide. Within a wheat strip, 3 rows of wheat were sown. Prairie strips were sown in October 2022 and a spring wheat was cultivated in the agroprairie treatments in spring 2023 before monitoring campaigns start in fall 2023. All prairies were harvested on October 10, 2023 with a ride-on mower for forage production and to facilitate the sowing of wheat. Localized tillage was applied in the wheat strips of agroprairies to prepare the seedbed. The soil was decompacted only in the wheat strips using a modified chisel (one 30 cm deep tine per strip) on October 16, 2023. Then, the uneven surface of the soil was tilled and levelled using a 45-cm-wide rotatory cultivator on November 17, 2023. Tilling temporarily reduced the size of the prairie strips from 37.5 to 30 cm, but prairie species recolonized this area during the growing season. Winter wheat was sown on November 25, 2023, at a density of 350 seeds m<sup>-2</sup>. No fertilizers and no pesticides were applied to be able to observe only the effects of prairie strips on wheat disease development and wheat belowground microbiome. Prairie strips were managed by mowing. In addition to fall mowing in October 2023, two more cuts were made during the experiment: in March 2024 and July 2024. On each date, cuts were made uniformly across the prairie treatments, 5 cm above the soil surface. Biomass was removed after the July 2024 cuts, whereas it was left in place following the March 2024 cut. The forage from the March 2024 cut was distributed as evenly as possible across the prairie strips. The wheat in agroprairie was systematically compared to a control treatment, that was wheat grown in monocrop (Figure S.1). To assess the impact of wheat-prairie strip intercropping on the STB development, two complementary experiments were carried out.

### 2.3. Experiment 1: effect of agroprairies on STB incidence and severity under natural epidemic conditions

#### 2.3.1. Design of agroprairies treatments

The different agroprairies treatments were obtained through the combination of a given prairie plant community and a given wheat cultivar. Two different prairie plant communities, named A and B, were made by grouping 15 plant species. The first plant community assembled consisted of species with high specific leaf area (SLA) and low leaf dry matter content (LDMC), so a priori more “acquisitive” species (Table 1). On the contrary, the second plant community assembled consisted of species with low specific leaf area (SLA) and high leaf dry matter content (LDMC), so being a priori more “conservative” species (Table 1). Each plant community were composed of species with contrasting growth form (grasses, legumes and other forbs, Table 1). Species

**Table 1**

Description of the four prairie types used in the agroprairie experiments and the relative abundance of sown species (% of seed mass).

	Functional type	Species name	Abundance of legumes when sowing		
			low	high	
a priori known as acquisitive species	Forbs	<i>Bellis perennis</i>	0.3	0.2	
		<i>Centaurea jacea</i> gr.	2.6	2	
		<i>Crepis biennis</i>	1.4	1	
		<i>Silene dioica</i>	0.8	0.5	
		<i>Veronica hederifolia</i>	5	3	
		Legumes	<i>Medicago lupulina</i>	3	30
			<i>Trifolium campestre</i>	1.5	0.2
			<i>Trifolium repens</i>	0.5	20
		Grass	<i>Agrostis capillaris</i>	1	0.5
	<i>Arrhenatherum elatius</i>		7	5	
	<i>Dactylis glomerata</i>		20	9	
	a priori known as conservative species	Forbs	<i>Holcus lanatus</i>	23	12
			<i>Lolium perenne</i>	16	9.7
			<i>Poa trivialis</i>	8	2
			<i>Schedonorus pratensis</i>	10	5
Legumes			<i>Carum carvi</i>	3	2.2
			<i>Centaurea jacea</i>	1.5	1.3
			<i>Daucus carota</i>	1.3	1
			<i>Knautia arvensis</i>	2.5	2.2
			<i>Plantago lanceolata</i>	2.9	1.5
			<i>Silene latifolia</i>	1	0.8
Grass			<i>Silene nutans</i>	0.4	0.3
			<i>Lotus corniculatus</i>	2	15
		<i>Medicago sativa</i>	1	10	
Legumes		<i>Trifolium pratense</i>	2	25	
		<i>Bromopsis erecta</i>	16	10	
	<i>Festuca ovina</i> gr.	15	5		
	<i>Festuca rubra</i>	37	15		
	<i>Phleum pratense</i>	10	8		
	<i>Poa pratensis</i>	5	2.8		
	<i>subsp. pratensis</i>				

selection was based on trait values gathered from the TRY database (Kattge et al., 2020). Three legume species were selected to be in each plant community, and represented either 5% or 50% of the seeds sown mass as to get the two plant communities divided in two sub-groups, named A1, A2, B1 and B2, with low or high legume abundance (Table 1). Near to the peak biomass, observations of species presence (on April 23, 2024) and leaf traits measurements (on May 28, 2024) enabled us to check the correspondence between what was sown and what was actually growing (see 2.3.2).

Four cultivars of winter wheat (*Triticum aestivum*) were chosen among a panel of 39 cultivars that were phenotyped for 15 functional traits, including aboveground and belowground traits (Dubs et al., 2023). The four cultivars selected were chosen to differ in terms of yield potential, maximal height, and investment in aboveground (relative growth rate, RGR) and belowground growth (specific root length, SRL and root dry matter content, RDMC) (Table S.2). Two landrace cultivars (high maximal height) were finally selected: “Rouge de Bordeaux” (high SRL, RDMC), “Barbu de Champagne” (high SRL), as well as to two elite

cultivars (high yield): "Rubisko" (high SRL), "Skerzso" (high RGR, RDMC)). These four cultivars also varied in their sensitivity to STB (Table S.2).

Overall, sixteen different agroprairie treatments were studied, resulting from the combination of four prairie plant communities and four wheat cultivars. We added four control treatments with the four wheat cultivars as monocrops (pure stand). All treatments were replicated four times in a randomized complete block design made of eighty plots in total (Figs. 2A, 2D), and this experiment was conducted without irrigation.

2.3.2. Biomass, height and traits measurements of intercropped prairie strips

Biomass measurement of the intercropped prairies was done on July 15, 2024. Two linear meters of prairies on both sides of a central wheat strip where cut in each plot, 5 cm from the base. Edge effects were minimized by always sampling the second and third out of four prairie strips within each plot. All the samples were then dried in an oven at 60°C for 48 h. The prairie biomass was weighted to evaluate the dry matter production. Vegetation heights were measured in the field on June 10, 2024 with a graduated measuring stick and two repetitions per plots. To consider plant community dynamic and possible species

invasion in the prairie, a botanical survey was conducted at the maximum biomass production and each present species in each experimental plot on April 23, 2024. Moreover, to take into account species traits plasticity in response to the growth conditions, a total of 10 individuals of each species per treatment were phenotyped for four morphological and one chemical functional traits (i.e., height, leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), and leaf N content (LNC)), following standardized procedures as described in Pérez-Harguindeguy et al., (2013). LA, SLA, LDMC, and LNC were measured on plant material collected on May 28, 2024, and the height of each species was measured on June 13, 2024, near to maximum plant biomass. Finally, the community weighted mean trait values were calculated for each functional trait as proxies of the functional structure of the plant community, based on the botanical survey conducted for each subplot.

2.3.3. Wheat height and nitrogen concentration of the upper leaves (Nup) measurements

Wheat vegetation heights were measured at the field with a graduated measuring stick on June 10, 2024 just before leaf senescence with 2 repetitions per plots. Nitrogen concentration of the upper leaves (Nup) of wheat was measured on April 22, 2024 at the Zadok stage 32. Six

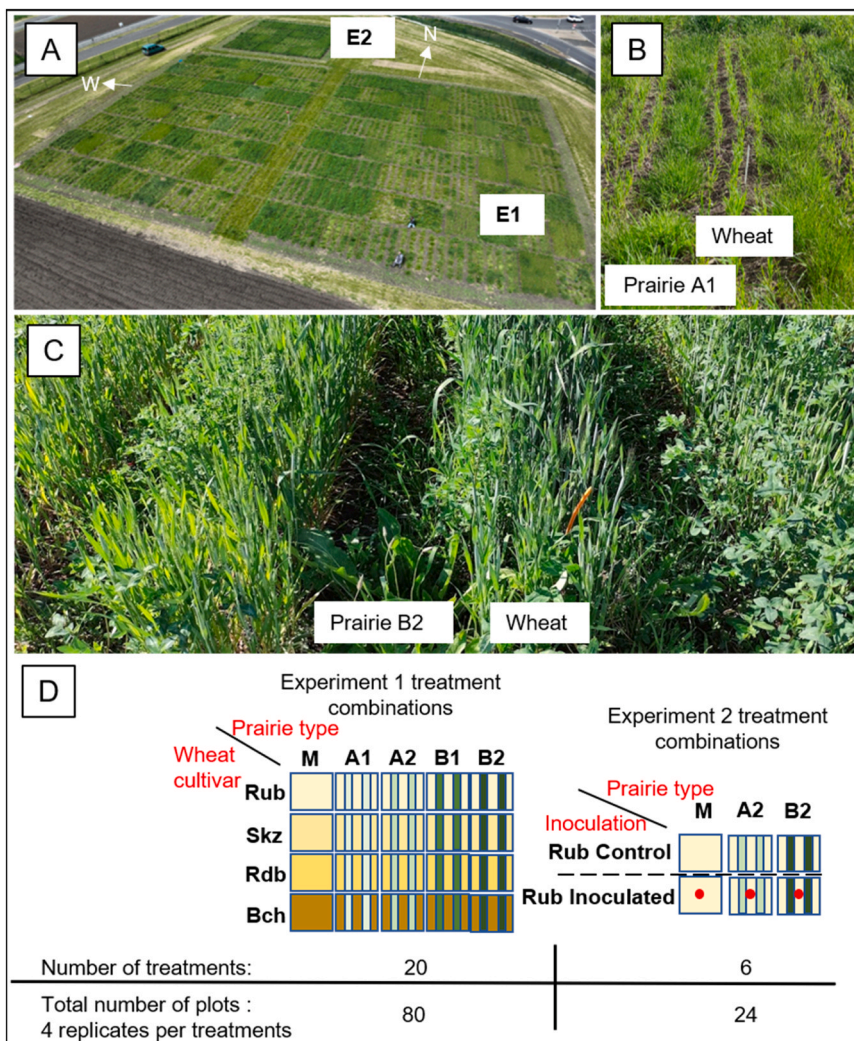


Fig. 2. Aerial view of the two experiments at INRAe of Clermont Ferrand (experiment 1: E1 and experiment 2: E2). Orientation (North and West) are indicated by the white arrows (A). Agroprairie including Rubisko at tillering stage and prairie A1 (high grass and low legume cover) (B), Agroprairie including Rubisko at bolting stage and prairie B2 (low grass and high legume cover) (C). Description scheme of the treatment combinations in experiments 1 and 2. Rub, Rubisko; Skz, Skerzso; Rdb, Rouge de Bordeaux; Bch, Barbu de Champagne. M, Monocrop; A1, A2, B1 and B2, type of prairie combined with wheat (D).

wheat leaves were randomly selected on each plot, and cut on 15 cm from the extremity of leaf. Samples of dried wheat leaves were ground in a vibrating ball mill and analyzed for their “bulk” elemental composition (%C and %N concentrations) using an Elemental Analyzer (Elementar Unicube CN Trace).

#### 2.3.4. Disease scoring

To quantify the STB development across the different treatments, disease assessments started at the second node stage, 151 days after sowing (April 24, 2024, Zadok stage 32). Then, notations of leaf incidence and severity were visually assessed on four notations dates: May 17, 2024, May 27, 2024, June 10, 2024 and June 25, 2024, corresponding to 174, 184, 198 and 213 days after sowing. At 213 days after sowing, the leaves were largely senescent, which prevented accurate quantification of disease necrosis. Therefore, this time point was excluded from the analysis.

Measurements were made on two randomly chosen linear meters per plots. To determine the STB incidence, the number of symptomatic plants was counted per linear meter. This number was divided by the mean plant density measured on the same plot after wheat emergence in February 2024, and expressed in percentage. Disease severity was visually estimated as the mean percentage of necrotic leaf area per linear meter. Both incidence and severity were assessed on the three uppermost leaf layers separately. For each plot and assessment date, two replicate measurements corresponding to the two randomly chosen linear meters were averaged per leaf layer to obtain a single incidence and severity value at the plot level. Although the final three leaves were monitored to capture the full disease development process, only the results for the last two leaves are presented for simplicity, as their symptoms have the greatest impact on wheat yield (Shaw and Royle, 1989). STB symptoms were rare on the flag leaf, which is why we focused on the results from the second leaf layer (Figure S.2).

Disease incidence and severity on the second leaf layer gradually increased until flowering and the onset of leaf senescence. As the assessment date of 198 days after sowing coincided with the end of flowering, this time point was selected for all analyses in Experiment 1 to represent the period of maximum disease impact. To compare the effect of agroprairies with the corresponding wheat monocrop control for each cultivar, log response ratios (log RRs; Hedges et al., 1999) were calculated for disease incidence and severity on the second leaf below the flag leaf. Log RRs were computed as  $\log(X \text{ in agroprairies} / \text{mean}(X \text{ in monocrop}))$ , where X is the incidence or severity for each agroprairie replicate, and mean X is the average incidence or severity of the four replicates in each monocrop. A logRR of  $< 0$  indicates a lower STB incidence or severity in agroprairie compared to monocrop.

To confirm the relevance of using the results from 198 days after sowing for analysis, log RRs were also calculated using the area under the disease progress curve (AUDPC), which accounts for disease effects throughout wheat development. The AUDPC (Campbell and Madden, 1990) was calculated for both incidence and severity separately as:

$$AUDPC = \sum_{i=1}^n \frac{1}{2} (X_i + X_{i-1})(t_i - t_{i-1})$$

where  $X_i$  and  $X_{i-1}$  represent the incidence and severity during the last and previous survey, respectively.  $t_i$  and  $t_{i-1}$  indicate the time of the last survey and the time of the previous survey, respectively.

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$$\text{Disease index} = \frac{\sum(\text{number of diseased leaves in each level} \times \text{value of the corresponding level})}{\text{total leaf number investigated} \times \text{value of the highest level}} \times 100$$


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#### 2.4. Experiment 2: effect of agroprairies on STB dynamics after inoculation and on the wheat rhizosphere microbiome

In this experiment, the most STB-sensitive cultivar, Rubisko, considered fairly sensitive (Table S.2), was grown both as a monocrop and in agroprairie designs, intercropped with two prairie plant communities (A2 and B2), which were expected to exhibit the greatest contrast in aerial architecture. An inoculated treatment and a control treatment without inoculation were implemented. A total of six treatments were studied, repeated four times in a randomized complete block design for a total of twenty-four plots (Figs. 2A, 2D).

##### 2.4.1. Inoculum preparation and field inoculation

*Z. tritici* strain IPO 09415 was transferred onto a YG liquid, incubated at 17°C under a 140 rpm agitation during seven days. 200 µl of this suspension was streaked onto YPD agar, and also incubated at 17°C during four days. The fungal material was transferred to a 0.05% tween water to obtain a concentrated spore suspension, without filtration. The suspension was then diluted in water to obtain a concentration of  $2.10^6$  spores/mL, corresponding to a high disease pressure level. Finally, the spore suspension was stored into a milk-glycerol mixture at -20°C. The inoculation took place on May 16, 2024, at flowering stage with no windy conditions. The inoculation zone was in the center of the plots, covering an area measuring  $2 \times 1.75$  m to avoid any dispersal to neighboring plots. The inoculum was applied evenly over the canopy using a hand-carried spray boom (2 m wide with 8 nozzles) connected to a tractor-mounted supply tank. The boom was maintained 30 cm above the wheat canopy, and the inoculum was regularly homogenized throughout the application. In total 145 mL of solution was sprayed per plots. Throughout Experiment 2, the plots were irrigated using sprinklers from June 6, 2024 to July 7, 2024 to maintain adequate humidity levels and ensure proper development of STB.

##### 2.4.2. Disease scoring

In the Experiment 2, a very large quantity of spores was sprayed on the inoculated plots, causing the disease to develop rapidly on all plants and leaves. This contrasts with natural epidemic conditions, where disease development is typically slower and does not necessarily affect all leaf stages. We therefore aimed to precisely quantify disease dynamics using an approach differing from Experiment 1.

For this purpose, twenty-five leaves per plots were randomly chosen and assessed in all treatments (inoculated vs. non-inoculated; two agroprairie types vs. monocrop). Three disease notations of incidence and severity were then conducted visually at the field on May 29, June 7 and June 17 2024, corresponding to 186, 194 and 205 days after sowing. One leaf sampling and visual notation on scanned leaf were done on June 14 only on inoculated plots. STB was evaluated according to a standard six-level grading system based on the leaf area covered with spores: 0 = no spore spots; 1 = <5% of the leaf area; 2 = 6–25% of the leaf area; 3 = 26–50%; 4 = 51–75%; and 5 = >75% of the leaf area (Hanouike, 1986). This scoring method allowed us to calculate the disease index, a metric that accounts for both disease incidence and severity (Zhang et al., 2025), which was calculated for each observation date using the following formula:

To integrate disease progression over time following inoculation, the AUDPC was also calculated for disease index using the same formula presented above, where  $X_i$  and  $X_{i-1}$  represent the disease index during the last and previous survey.

#### 2.4.3. Rhizosphere sampling and DNA extraction

Rhizospheric samples were taken before inoculation on April 24 from all the plots of the experiment 2 during the booting period. To extract the soil rhizosphere from wheat plants, four composite wheat plants per plots were uprooted carefully and shaken to only keep the adherent soil on the roots. To extract the soil rhizosphere from prairie plants, four composite cores were collected in the strips as close as possible to the sampled wheat plants using a root auger (10 cm wide and 15 cm deep), and then pooled. The protocol to separate root and rhizosphere samples was adapted from Simmons et al., (2018). Roots samples were washed with buffer solution (900 mL sterile H<sub>2</sub>O + 6.075 g KH<sub>2</sub>PO<sub>4</sub> + 7.875 g K<sub>2</sub>HPO<sub>4</sub> + 900 µL Triton X-100) and a firmly shaken with a sonification. Roots were then separated from the solution, and washed with sterilized water. In the same time, rhizospheric soil solution was centrifugated. Then supernatant was removed for keeping only rhizospheric soil. Each sample was immediately stored at -80°C for DNA extraction. Later, DNA was extracted using the Fast DNAtm SPIN Kit for Soil (MP Bio-medicals) according to the manufacturer's instructions. Briefly, 200 mg of each sample was grinded in detergent to disrupt and dissolve membranes. After centrifugation with a solution to precipitate proteins, the supernatant was mixed with a DNA-binding silica matrix. The remaining solution was washed and filtered to obtain only the product of extraction in DNase- and Pyrogen-Free Water. The purity and concentration of the extracts were measured with a NanoDrop™ spectrometer (Ozyme).

#### 2.4.4. PCR amplification and 16S and ITS-28S rRNA gene amplicon sequencing

The 27 F (5'- AGRGTTYGATYMTGGCTCAG -3') and 1492 R (5'- RGYTACCTTGTTACGACTT -3') was used to amplify the V1-V9 variable region of the bacterial 16S rRNA gene (PacBio, 2022) and the ITS1catta|| (5'-ACCWGGCGARGGATCATA-3') and LR5.TW14ngs (5'-TCCTGAGG-GAAACTCG-3') was used to amplify the ITS-28S of fungus rRNA gene (Tedesoo and Anslan, 2019; Tedesoo and Lindahl, 2016). Both the forward and reverse of the two pairs of primers were tailed with sample specific PacBio barcode sequences to allow for multiplexed sequencing. The KAPA HiFi Hot Start DNA Polymerase (KAPABiosystems) was used for PCR amplification. For 16S rRNA amplification, after initial denature step (95°C for 3 min), 23 cycles of PCR amplification, with denaturing at 95 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 60 s. For ITS-28S amplification, after initial denature step (95°C for 15 min), 30 cycles of PCR amplification, with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s. Amplified DNAs pool was processed with the SMRTbell Express Template Prep Kit 2.0 (PacBio, USA), following the Procedure & Checklist - Amplification of Full-Length 16S Gene with Barcoded Primers for Multiplexed SMRTbell® Library Preparation and Sequencing protocol (Part Number 101-599-700 Version 04, PACBIO). The libraries were prepared and sequenced using long-read sequencing on a PacBio Sequel II at the Gentyane platform (INRAE, France).

## 2.5. Statistical analysis

### 2.5.1. Experiment 1

All statistical analyses were conducted with R 4.5.1. (R Core Team, 2024).

First, a non-binomial generalized linear model was applied to raw incidence (number of infected plants) data to compare the different incidence results between wheat cultivars in monocrop. For this, the glmmTMB function from the glmmTMB package (Brooks et al., 2017; version 1.1.12) was used to test the effects of the block, the notation repetition, and the wheat cultivar. The total number of plants on a linear

meter was included as an offset in the model, the model family was "nbinom2", using the parameter ziformula= ~1 to consider zero inflation. To validate this model, the plot function from the DHARMA package (Hartig, 2016) was used to test the homogeneity of the variances, the normality and the independence of the residuals from the model. The effect of cultivars on maximum height and leaf nitrogen concentration in monocrop was also tested using linear models, with only the cultivar as an effect. To ensure that the four prairie plant communities were differentiated according to their growth strategy and their percentage of legumes, a PCA was carried out on mean SLA, LDMC, LA, LNC and height described in Material and methods 2.3.2 using the PCA function in the FactorMineR package (Husson et al., 2025; version 2.12). To quantify the effect of agroprairies on STB development, linear models were fitted using the lm function on two types of log response ratios (log RR): the log RR of incidence and severity AUDPC, and the log RR of incidence and severity assessed at 198 days after sowing. The effect of the categorical variables describing the intercropped prairie strips (A1, A2, B1, B2) was tested, with the block also included, in order to quantify the effect of wheat-prairie intercropping independently. Since the categorical models produced similar trends for both types of log RR, with much lower variability at 198 DAS, the values from this date were retained for further modelling. A second linear model was then fitted to investigate the potential drivers of the effects of wheat-prairie strip intercropping on the log RR. This model was built following a forward exploratory selection logic: starting with categorical variables such as intercropped prairie type (A1, A2, B1, B2) and wheat cultivar, and then decomposing them into continuous quantitative variables characteristic of these categories. The factors tested included wheat cultivar, wheat height, nitrogen concentration of the upper leaves (Nup), prairie height, prairie biomass, prairie specific leaf area (SLA), and the proportion of legumes in the associated prairies. Interactions between these variables were also tested. Because these variables reflect different aspects of plant functioning, they were measured at the developmental stages most relevant to their biological meaning. Leaf samples, for example, were collected in mid-May, at peak biomass, in order to best capture the plants' resource acquisition strategy at their maximum growth stage. In contrast, dry biomass represents an integrated indicator of plant growth over time and can therefore be collected later in the season without loss of information. As a result, traits measured in mid-May and biomass measured in early July were used as proxies of prairie growth and resource acquisition strategy in our models, even though they were not recorded at exactly the same time as the disease assessment performed at 198 days after sowing.

All tested models, along with their corresponding Akaike Information Criterion (AIC) values are detailed in Table S.4. The model with the lowest AIC was selected. Accordingly, one model for incidence and two models for severity were considered competing models with the one with the lowest AIC criteria, as they had a  $\Delta AIC < 2$ . In such cases, the model showing the best residual homogeneity was retained. To assess multicollinearity among explanatory variables in the selected model, we computed the Generalized Variance Inflation Factor (GVIF) and used the adjusted form  $GVIF^*(1/(2 \cdot Df))$ , detailed in Table S.5, which accounts for the degrees of freedom associated with each variable (Fox and Weisberg, 2018).

### 2.5.2. Experiment 2

The effect of wheat-prairie strip intercropping on disease dynamics in inoculated plots was quantified using the AUDPC as the response variable, along with the effects of the associated prairie type and of the block. The same approach was applied to non-inoculated plots, using log RR as the response variable as indicated above. For all the selected models, residuals normality was checked using the Shapiro test, homoscedasticity using the Bartlett test and the independence of the residuals was checked graphically. Then analyses of variance were performed using the car package. For linear models with only fixed effects, Type II ANOVAs were performed. Then, post-hoc analyses of treatments were

conducted using Tukey's HSD test. In the case of the analyses of logRR as the response variable, a one-sample Wilcoxon test was used to assess which treatment had a logRR significantly different than zero. For linear models including interactions, Type III ANOVAs were applied.

Concerning microbial analysis, after demultiplexing and removal of the barcodes, the reads were processed with the package DADA2 (Callahan et al., 2016; version 1.36.0.). The primers were removed, and the reads were filtered to keep only fragments between 1000 and 1600 bp. The sequences were dereplicated and the errors were corrected using the learnerrors function of the package. The sequences were denoised and taxonomically assigned up to the species level with a 97% confidence threshold using SILVA database for bacteria and UNITE database for fungi. For the microbiome analysis, the resulting data were treated with the phyloseq package (McMurdie and Holmes, 2013; version 1.41.1). The sequences were filtered to keep only bacterial and fungal sequences. Alpha diversity analysis was assessed using the Shannon and inverse Simpson indices.

Beta diversity analysis were performed using PCOA with the Aitchison distance. Then PERMANOVAs were then performed across plots, using plot as the unit of observation with the adonis2 function from the vegan package with 999 permutations, stratified by block. Permutation reproducibility was guaranteed by setting the random seed to 123. To ensure homogeneity of dispersion, a test for multivariate homogeneity of group dispersions was performed using the betadisper function from the vegan package (Oksanen et al., 2013; version 2.7.1).

To investigate the relationship between rhizospheric microbiome diversity (alpha and beta diversity) and STB development, two Principal Component Analyses (PCAs) were performed using the same package described above. The first PCA included the Shannon index, the values of the first PCoA axes for both fungi and bacteria, and AUDPC values under inoculated conditions. The second PCA included the first PCoA axes for fungi and bacteria, along with STB incidence values at the final scoring date under non-inoculated conditions.

To investigate taxa involved in pathogen inhibition and induced systemic resistance (ISR), a list of bacterial and fungal genera, and species within these genera, was compiled. This list was based on literature reviews describing the general role of the rhizosphere microbiome in plant disease control. This list was used as a basis for analyzing the microbiome dataset (Table S.6; H. Liu et al., 2020; Pieterse et al., 2014; Qu et al., 2020; Yang et al., 2023; K. Yu et al., 2019). From this list,

we examined whether these taxa were present in our dataset. For those detected, the effect of the different treatments on their abundance was analyzed per taxa, following verification of the assumptions required for linear modeling using the methods described above. To meet these assumptions, data were transformed when necessary using the Boxcox function of the MASS package (Ripley et al., 2025, version 7.3.65). When assumptions were not met, even after transformation, data were analyzed using a Kruskal-Wallis test followed by post hoc Wilcoxon tests with Bonferroni adjustment.

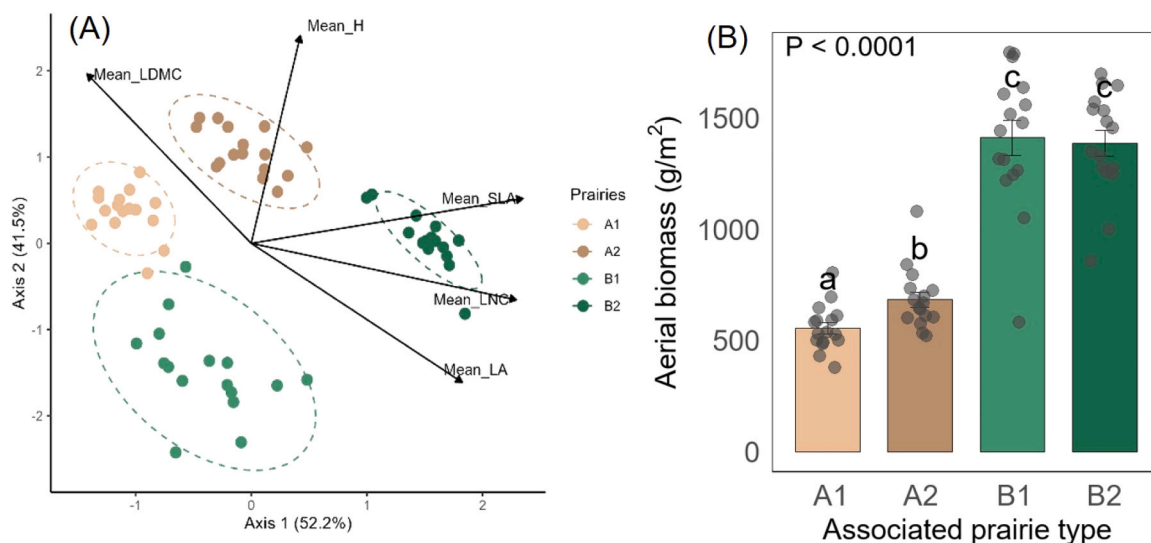
### 3. Results

#### 3.1. Experiment 1

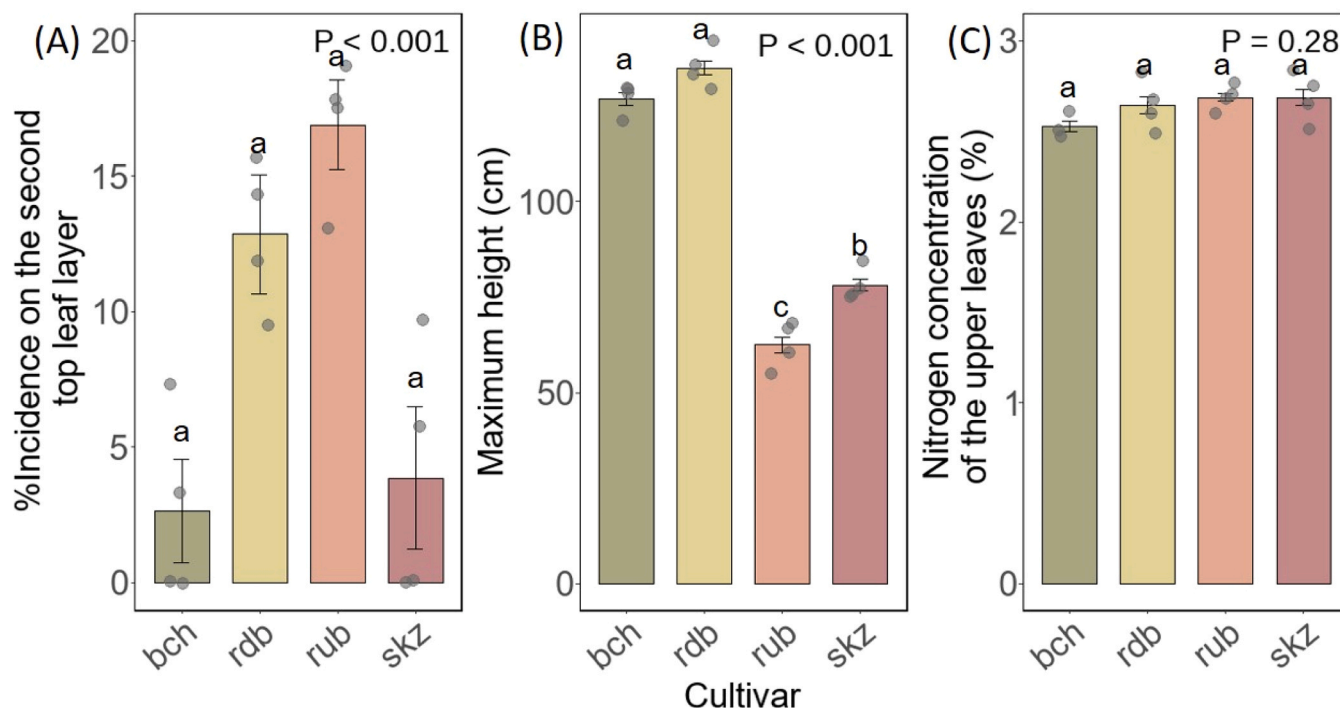
##### 3.1.1. Prairies growth and traits

At peak biomass, the species composition of the four prairie types differed and generally reflected the sown species, even though not all sown species were present and percentage cover did not always match the proportions of sown biomass (Table S.3). Prairies A1 and A2 were dominated by grasses (>88%). Prairie B1 exhibited a higher forb cover (~25%) and an intermediate legume cover (~28%) compared to other prairie types. Prairie B2 was characterized by a predominance of legumes (64%) (Table S.3). The four prairie types also differed functionally: A1 and A2 were characterized by high leaf dry matter content (LDMC) (Fig. 3A), whereas B1 and B2 exhibited high leaf area (LA). Prairie B2 additionally displayed high specific leaf area (SLA) and leaf nitrogen content (LNC) (Fig. 3A). This result highlights a discrepancy between the expected functional traits of the prairies at sowing and those observed later in the field. Contrary to our expectations, A1 and A2 exhibited traits more consistent with a "conservative strategy," whereas B1 and B2 displayed trait syndromes closer to an "acquisitive strategy".

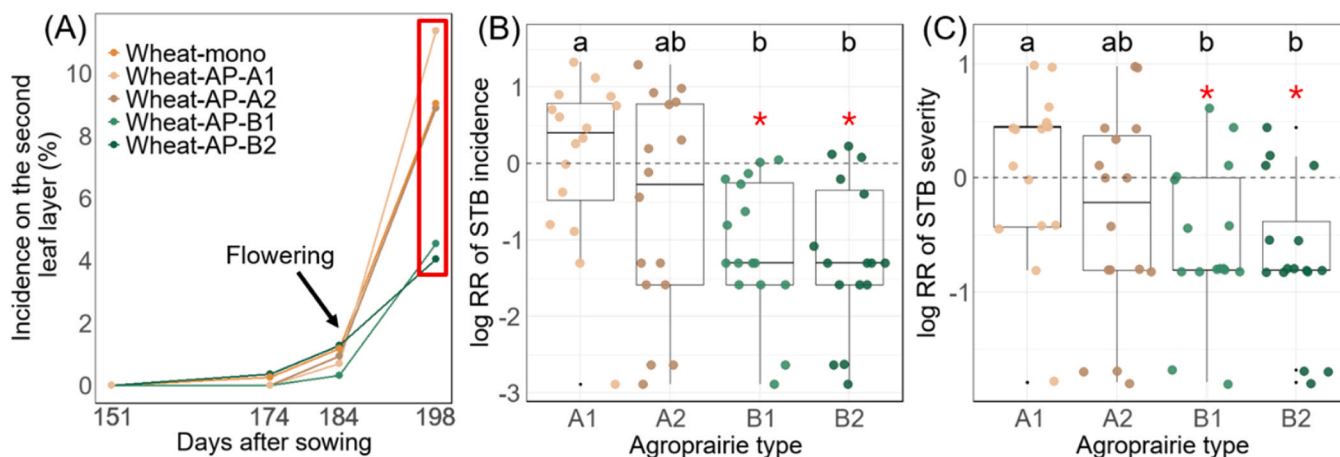
These differences in species and traits between the four functional prairie types led to contrasted aerial biomasses, with the lowest average biomass of 556 g/m<sup>2</sup> for prairie A1, followed by prairie A2 with 684 g/m<sup>2</sup>, and prairies B1 and B2 with 1400 g/m<sup>2</sup> (p-value < 0.0001; Fig. 3B). A nonlinear relationship was observed between prairie biomass and legume proportion: prairie biomass increased up to approximately 30% legume cover, beyond which prairie biomass showed no further increase (Figure S.4B).



**Fig. 3.** Principal component analysis of the mean value of functional traits in prairies strips, as a function of prairie type (A1, A2, B1, B2). SLA, Specific leaf area; LNC, Leaf nitrogen content; LA, Leaf area; LDMC, Leaf dry matter content; H, Height. Measurements were taken in May 2024 near to the peak biomass (A). Mean aerial biomass between the different associated prairie types. Biomass was sampled in July 2024. Values are expressed as the mean  $\pm$  standard error ( $n = 16$ ). Different lowercase letters represent significant differences between treatments at  $p$ -value < 0.05 (B).



**Fig. 4.** Septoria tritici blotch (STB) incidences levels on the second top leaf layer (A), maximum height (B) and nitrogen concentration in the upper leaves (C) in monocrops of the four different wheat cultivars. bch, Barbu de Champagne; rdb, Rouge de Bordeaux; rub, Rubisko; skz, Skerzto. The p-values indicate the results of the cultivar effect from the negative binomial model (A) or linear models (B, C). Different letters indicate significant differences between the wheat cultivars. Mean values and standard errors are shown (n = 4).



**Fig. 5.** Evolution of the STB incidence on the second top leaf layer between treatments. Wheat-mono, Wheat monocrop; Wheat-AP, Wheat in agroprairies (A). Log response ratios (logRR) of STB incidence (B) and severity (C) measured on the second leaf layer, 198 days after sowing (DAS), in the different types of agroprairies (A1, A2, B1, B2). B2, agroprairies with the highest legume rate; B1, agroprairies with higher forbs and medium legumes rate, A2 and A1, highest grass rate and lower legumes cover. Different lowercase letters represent significant differences between treatments at p-value < 0.05. Red stars indicate the values significantly lower than 0 (p-value < 0.05).

### 3.1.2. Wheat cultivar STB sensitivity, growth and leaf nitrogen content

The wheat cultivar Rubisko was the most infected cultivar, with 17% of plants on average having STB symptoms. The cultivar Rouge de Bordeaux had 13% of infected plants on average, while Skerzto and Barbu de Champagne had only 4 and 3%, respectively (Fig. 4A). As expected, wheat cultivars exhibited contrasting plant heights but similar nitrogen concentrations in the upper leaves (Figs. 4B, 4C).

### 3.1.3. The effect of agroprairie design on STB incidence and severity in natural epidemic conditions

Regarding disease progression in wheat, the incidence of STB did not increase much until the wheat flowering stage, with an average

incidence of no more than 3% (Fig. 5A). At 198 days after sowing (DAS), at the end of flowering stage and before leaf senescence began, the logRR of incidence and severity differed significantly between treatments (Figs. 5B and 5C; p-value = 0.002 and p-value = 0.01, respectively). At this date, the incidence of the disease varied between, on average, 4% and 11% of infected plants, with wheat grown in monocrop and agroprairies A1 and A2 showing more symptoms than wheat grown in agroprairie B1 and B2 (Fig. 5A). Furthermore, the logRR of the incidence was significantly lower than zero for agroprairies B1 and B2 without significant difference between the two prairie types (Fig. 5B; p-value < 0.001) indicating a decrease of STB incidence in agroprairies B1 and B2 compared to monocrop, with an average incidence reduction of

52% across all cultivars. In contrast, the agroprairies A1 and A2 had no significant effect on the log RR of disease incidence. However, A2 exhibited more intermediate values and greater within-group variability than A1. Regarding disease severity, agroprairies B1 and B2 showed significantly negative logRR values (Fig. 5C; p-value = 0.004 and p-value = 0.001, respectively), corresponding to an average severity reduction of 41%. Similar trends, though with greater variability, were observed for the logRR of incidence and severity area under disease progress curve (AUDPC), indicating that values at 198 days after sowing are representative of disease dynamics (Figure S.3; p-value = 0.002 and p-value = 0.02). However, severity AUDPC in agroprairie B2 did not differ significantly from A1 or B1, in contrast to the result at 198 days after sowing (Figure S.3).

Among the candidate models, the best-supported model explaining differences in STB incidence and severity between agroprairie treatments included effects of prairie biomass and SLA (Table 2). Higher prairie biomass and higher SLA were associated with a lower logRR, corresponding to a greater disease reduction in the agroprairie compared to monocrop (Figure S.4 A). A significant interaction between legume percentage and prairie biomass was detected in explaining the log RR of STB incidence, with the interaction showing a positive effect on logRR (p-value = 0.03). This indicates that, with similar biomass, prairies with more legumes tend to increase the STB incidence in agroprairies. No effect of this interaction has been observed for logRR severity results (Table 2).

Compared to monocrops, wheat grown in agroprairie design showed higher nitrogen concentration in the upper leaves across all cultivars, except for Rouge de Bordeaux (Figure S.5 A; p-value < 0.05). The effect of strip intercropping on plant height depended on the wheat cultivar (Figure S.5B; p-value < 0.05). For instance, Rubisko was significantly taller in strip intercropping with prairie B2 than in monocrop (Figure S.5 C; p-value < 0.05). However, none of these phenotypic variables and interaction between wheat cultivars and the agroprairie design were retained as predictors in the candidate model set (Table S.4).

## 3.2. Experiment 2

### 3.2.1. The effect of agroprairie design on STB progression after inoculation

In the control treatments (not inoculated), an average of 41% and 28% of the wheat plants showed STB symptoms, in monocrop and agroprairies settings (Fig. 6A). As in the first experiment, wheat intercropped with prairie strip B2 had significantly lower disease incidence and severity than wheat intercropped with prairie strip A2 (p-value < 0.05), although both prairie strips reduced disease compared to the monocrop situation (Figure S.6).

In inoculated treatments, 100% of the wheat plants were infected by STB (Fig. 6A), and necrosis represented an average of 80% of the noted leaf surface at the end of the growing season (Fig. 6B). In contrast to not inoculated treatments, the agroprairie A2 enabled better disease

regulation compared to agroprairie B2. In inoculated conditions, AUDPC, here calculated as the integration of incidence and severity on the two upper leaves to summarize overall disease progression, was reduced by 18% compared to monocrops (Fig. 6C).

### 3.2.2. Rhizosphere microbial diversity of the prairie plant communities

The bacterial phyla identified in the prairie rhizosphere samples were mainly represented by *Proteobacteria*, *Acidobacteriota* and *Bacteroidota* (Figure S.7 A). The fungal phyla of soil rhizosphere were dominated by *Ascomycota* and *Basidiomycota* (Figure S.7B). No significant differences were observed between the two intercropped prairie plant communities for the rhizosphere fungal and bacterial alpha diversity (Shannon and InvSimpson indices) (Figure S.8B). Regarding rhizosphere beta diversity, we observed significant differences of fungal and bacterial communities between prairie A2 and B2 (Figure S.9 A, p-value < 0.001).

### 3.2.3. Changes in the wheat rhizosphere microbiome between agroprairie treatments

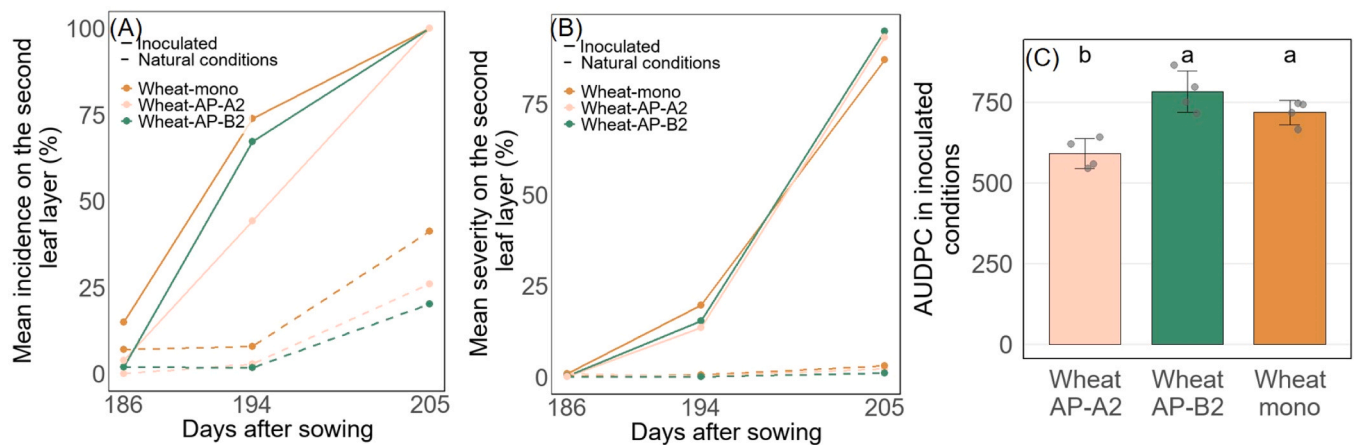
The bacterial phyla identified in the wheat rhizosphere were similar to those in the intercropped prairie strip (Figure S.7 A). No significant differences were observed for the rhizosphere fungal and bacterial alpha diversity (Shannon and InvSimpson indices) between treatments (Figure S.8 A). Concerning wheat rhizosphere microbiome beta diversity, differences in bacterial communities were found in wheat grown in monocrop and in agroprairies (Fig. 7A, p-value = 0.003). Despite the differences in community structure, we did not detect any influence of microbial diversity indices (alpha or beta) on disease indicators (incidence, severity or AUDPC) under natural or inoculated conditions (Figure S.9B).

Our literature review identified 18 bacterial and 7 fungal genera involved in the plant induced systemic resistance or in pathogen inhibition effect (Table S.6). Our experiment revealed that 11 of these bacterial genera were present in the wheat rhizosphere (Fig. 7B, Table S.6). Among these genera, *Pseudomonas* was significantly more abundant in wheat grown in agroprairie A2 than in monocrop (Fig. 7C; p-value = 0.03). At the species level (Table S.6), *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas brassicacearum* and *Stenotrophomonas maltophilia* were present in our samples. Only *Pseudomonas brassicacearum* was significantly more abundant in the wheat rhizosphere when intercropped with prairie strip A2 (Figure S.9 C; p-value = 0.03). Although the disease development appeared to slow down with this agroprairie treatment, we could not find a significant correlation between disease development and *Pseudomonas brassicacearum* abundance (p-value = 0.9). Concerning fungi, the only genera identified in our samples that match those known to be involved in plant-induced systemic resistance or pathogen inhibition (Table S.6) was *Penicillium*. However, no significant differences in abundance were observed among our experimental treatments (Fig. 7C).

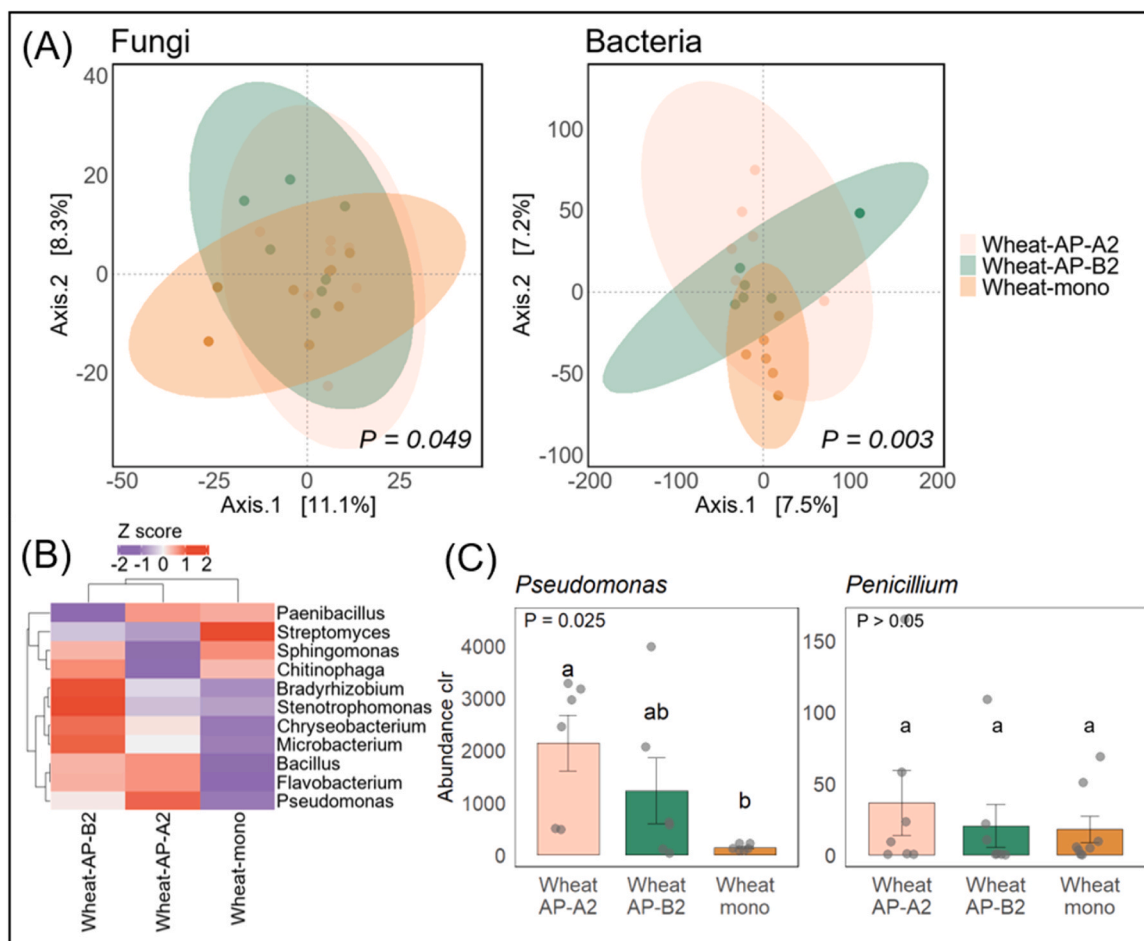
**Table 2**

Results of the selected linear models chosen with the AIC criteria explaining the logRR of the incidence and the severity of STB in different types of agroprairies. % Legumes: Percentage of legumes in the associated prairie; Prairie biomass: Total aerial biomass of the associated prairie ( $\text{g}\cdot\text{m}^{-2}$ ); SLA: Mean specific leaf area of the associated prairie ( $\text{cm}\cdot\text{g}^{-1}$ ). Significant p-values are indicated in bold.

	Response variables:								
	log RR of STB incidence values				log RR of STB severity values				
	adjusted R <sup>2</sup> = 0.36				adjusted R <sup>2</sup> = 0.29				
	df	Effect size	F df,53	p-value	df	Effect size	F df,53	p-value	
% Legumes	1	0.1	1.79	0.18	1	0.2	0.77	0.38	
Prairie biomass	1	-0.4	15.81	< 0.001	1	-0.4	11.27	0.001	
SLA	1	-0.3	4.52	0.04	1	-0.4	4.43	0.04	
Wheat cultivar	3		1.29	0.28	3		1.97	0.13	
% Legumes:Prairie biomass	1	0.3	5.28	0.03	1	0.3	3.17	0.08	
Block	3		4.97	0.004	3		3.27	0.02	



**Fig. 6.** Mean disease incidence (A) and severity (B) progression on the second leaf layer in inoculated and natural conditions. Area under disease progress curve in inoculated conditions, considering the disease index, calculated as a weighted sum of incidence and severity in inoculated conditions. Values are expressed as the mean  $\pm$  standard deviation ( $n = 4$ ). Treatments: Wheat-AP-A2, wheat in agroprairie A2; Wheat-AP-B2, wheat in agroprairie B2; Wheat-mono, wheat monocrop. Different lowercase letters represent significant differences between treatments at  $p$ -value  $< 0.05$  (C).



**Fig. 7.** Principal coordination analysis (PCoA) based on Aitchison distances of fungi and bacteria of wheat rhizosphere community structure (A). Heat map presenting the different bacterial genera observed in rhizosphere samples of the experiment 2, and which are known to be involved in plant induced systemic resistance and pathogen inhibition. A Z-score greater than 0 indicates enrichment of the bacterial genera (B). *Pseudomonas* (bacterial genera) and *Penicillium* (fungal genera) abundances in wheat rhizosphere between the different treatments. The abundances values are centered and log transformed. Values are expressed as the mean  $\pm$  standard error ( $n = 6$ ). Different lowercase letters represent significant differences between treatments at  $p$ -value  $< 0.05$ . These were chosen by performing a Wilcoxon test with the Bonferroni adjustment method. The relative abundance is expressed as centered log ratio (clr) (C). Treatments: Wheat-AP-A2, wheat in agroprairie A2; Wheat-AP-B2, wheat in agroprairie B2; Wheat-mono, wheat monocrop.

#### 4. Discussion

In this study, we hypothesized that *Septoria tritici* blotch (STB) could be regulated differently in agroprairie compared to monocrop designs, and within the different agroprairies depending on the wheat cultivar and the prairie plant community used. Using our experimental approach, we established four functional types of prairies that varied in species composition, and functional traits.

We anticipated that discrepancies would emerge depending on the effectiveness of specific regulatory mechanisms, among which is i) a barrier effect that limits the dispersal of pathogens; ii) modifications of host morphology and physiology; iii) alterations in host plant sensitivity resulting from changes in its rhizospheric microbiome (Fig. 1).

##### 4.1. Reduction of STB incidence and severity with agroprairies under natural epidemic conditions

An important finding of this study is the average reduction of 52% in STB incidence and 41% in STB severity observed in two of the four tested agroprairies (B1, B2) compared to monocrop under natural conditions of pathogen development (experiment 1). These observed reductions derive from the logRRs calculated at the end of the flowering stage, 198 days after sowing. The positive role of plant diversification for controlling STB has been studied in wheat cultivar mixtures (e.g. Kristoffersen et al., 2020; Orellana-Torres et al., 2022; Vidal et al., 2020), but it is comparatively less documented under open-field strip intercropping.

Another key finding of our study is the variable effect of the agroprairie design on STB severity and incidence depending on the prairie plant community used. We demonstrated that the reduction of STB severity and incidence were maximized in agroprairie treatments with high prairie strip biomass and mean specific leaf area (SLA) (Table 2). The role of the prairie biomass in explaining STB regulation in our models suggests the efficiency of the physical barrier effect in preventing the spore dispersal. This is supported by the fact that *Z. tritici* is a hemibiotrophic specific pathogen that can disperse horizontally via wind over long distances and vertically via rain (Suffert et al., 2011), and is therefore highly susceptible to the barrier effect (X. Liu et al., 2020; Ratnadass et al., 2012). This consistent barrier effect is further supported by the fact that its efficiency with high-biomass prairies was no longer observed in experiment 2 with disease inoculation above the canopy. The role of intercropping in spore dispersal interference was notably shown under controlled and inoculated conditions (Bannon and Cooke, 1998), where a wheat-clover intercropping system proved to reduce horizontal spore dispersal by 33% and vertical spore dispersal by 63% compared to a monocrop.

Furthermore, the agroprairie design consistently regulated STB incidence and severity across wheat cultivars, with no significant interaction between cultivar and agroprairie type. This consistency is particularly noteworthy because wheat cultivars have contrasted traits and these traits responded differently to intercropping with prairie strips (Figure S.5). Some of these traits such as plant heights and leaf nitrogen concentration are known to influence STB dynamics by modifying resource availability and microclimate for fungal development (Julian Maywald et al., 2023; Vidal et al., 2017) and one might have expected these traits to change the effect of prairie on STB in our experiment. For example, lower leaf nitrogen concentration in the Rouge de Bordeaux cultivar in agroprairies compared to monocrop may have constrained STB development, whereas increased plant height in agroprairies for the Rubisko cultivar could have facilitated the transfer of pathogens between wheat plants separated by prairie strips. However, despite these trait differences, they were not identified as primary predictors of STB incidence or severity in our analyses. The consistent effect of prairies could be due to the importance of the barrier effect in regulating the spread of the disease, a process that is relatively independent of wheat traits.

Finally, the interaction between biomass and legume abundance in prairie strips and its effect on STB incidence suggests hypotheses that were not directly tested in this study. For example, the percentage of legumes may affect microclimate, microbiome composition and resource availability, which may influence the STB development and wheat sensitivity (Boudreau, 2013; Duchene et al., 2017). However, the precise origin of this interaction remains unclear, and further research under more controlled conditions is needed to better understand this effect.

##### 4.2. Effect of agroprairies on STB progression after inoculation

Inoculation above the canopy with the fungal pathogen *Z. tritici* resulted in infection of all plants in both monocrop and agroprairie designs by the end of the growing season (Fig. 6A), with an average of 80% leaf exhibiting necrosis (Fig. 6B). Thus, the regulating effect of disease dispersion by agroprairies observed under non-inoculated conditions, especially in situations with high biomass prairie strips, was no longer operational. However, the AUDPC analysis revealed differences in the progression extent of the disease on wheat plants between treatments (Fig. 6C). Interestingly, the agroprairie A2 with low biomass prairie strips significantly slowed the progression of STB, reducing the AUDPC by 18% compared to the wheat monocrop (Fig. 6C). The A2 prairie strips produced over 50% less biomass than the B2 strips in the experiment 1 (Fig. 4B) and exhibited similar differences in experiment 2. These results suggest that mechanisms other than the physical effect of prairie biomass were at play, and that prairie biomass contributes to STB regulation in agroprairies only under certain disease pressure and infection levels.

##### 4.3. Increased beneficial microorganisms in the rhizosphere, while not correlated to STB indicators

In this study, we focused on the wheat rhizosphere microbiome, as it is particularly responsive to environmental changes induced by crop diversification and is considered to play a key role in the induction of plant defense mechanisms and pathogen suppression (H. Liu et al., 2020; Qu et al., 2020).

In our situations, the relative abundances of bacterial and fungal phyla observed in the wheat rhizosphere were consistent with those commonly reported in the literature (Trivedi et al., 2020). The distinct beta diversity of the microbial communities found in the agroprairie and monocrop treatments in experiment 2 (Fig. 7A) strengthened our general understanding of the influence of plant community composition and traits on microbial communities (Fig. 7A, Table 1). This observation is consistent with previous studies reporting shifts in the wheat rhizosphere or endophytic root microbiome when intercropped (Granzow et al., 2017; H. Yu et al., 2019) or in contact with certain weeds (Ricono et al., 2025). Interestingly in our case, the genera *Pseudomonas* was significantly more abundant in the wheat rhizosphere when intercropped in the A2 prairie strips (Fig. 7C). The disease-suppressing effects of *Pseudomonas* spp. are now well established. These bacteria can act as biocontrol agents through multiple ways, including antibiosis and the induction of systemic resistance (Höfte, 2021). For example, *P. brassicacearum* has been studied for its positive role on plant defense and global rhizosphere fitness (Liu et al., 2018) and for the production of antibiotics efficient against the development of various pathogens (Bahmani et al., 2021; Mandryk-Litvinkovich et al., 2017). Although we observed no effect on disease regulation under our field conditions with massive disease inoculation or in natural epidemic conditions, these changes in microbial communities are likely crucial for developing a more resilient growing environment for crops (Fadji et al., 2023; Fang et al., 2025).

#### 4.4. Research perspectives

Under our field conditions, two of the four tested agroprairies reduced the spread of STB under moderate epidemic pressure. We suggest that this result is primarily driven by a barrier effect, and is likely to vary depending on local climate and disease pressure. In our study, wind direction and strip width were not tested, so the following remain hypotheses for future investigation. We propose that strips oriented perpendicular to prevailing winds may better limit *Z. tritici* spore dispersal than strips aligned with the wind. Additionally, we hypothesize that width of the strips could influence the strength of the barrier effect. Future studies could explore these factors to determine the conditions under which prairie strips most effectively intercept pathogen spores.

From a technical standpoint, the most important obstacle to implement agroprairies is the lack of tools to enable differentiated management of cereal and prairie strips, whether for sowing, tillage, mowing, or mulching. One or more mowing operations are required to minimize competitive interactions between the prairie strip and the wheat, including potential encroachment into the wheat strip during the growing season. In addition, following wheat harvest, prairie species may further colonize the wheat strip. Localized tillage of the soil in the cultivated strip is therefore necessary to remove residual prairie vegetation and ensure adequate seedbed preparation for the subsequent crop. However, research is underway to develop effective tools that enable differentiated management of co-occurring strips including localized GPS-guided tillage and mowing (Carrière et al., 2024). Regarding the products resulting from this system, agroprairies could enable substantial production of species-rich forage for use as animal feed or green manure, while maintaining a cash crop production.

Furthermore, it is also important to note that in our non-inoculated experiment, disease pressure was moderate. In regions with higher natural disease pressure, the capacity of strip intercropping of wheat and prairie to reduce STB may be more limited, and the regulation effects observed in this study may not be as pronounced. Nevertheless, the interaction between agroprairie design and cultivar was not retained in the model selection, indicating that the effect we observed of some agroprairies designs (e.g. B1/B2) against STB are likely consistent across all cultivars. These results suggest that the protective role of certain agroprairies could potentially be extended to a broader range of cultivars and combined with other control strategies, such as the selection of resistance genes.

Although some variation in cultivar sensitivity was observed, the cultivars used in this study were not highly sensitive to STB (Table S.2). We therefore recommend conducting further experiments to assess both the effectiveness and limitations of wheat-prairie strip intercropping in reducing STB and other wheat pathogens impact under diverse climatic conditions, particularly when using highly sensitive cultivars.

On the other hand, we observed that plant traits indicative of growth strategy of the intercropped prairie (SLA) could be linked to STB regulation (Table 2). This highlights the importance of studying the role and distribution of plant functional traits in ecosystem functioning, particularly with regard to pathogen regulation (Le Bagousse-Pinguet et al., 2021).

Finally, our study highlighted the complexity of disentangling specific mechanisms (e.g., microbiome shifts) from more overarching processes (e.g., barrier effects) involved in disease reduction within a field study manipulating diversified agroecosystems. Progress in this field requires balancing field research, which captures macroscopic and systemic phenomena, with more targeted, mechanistic and reductionist studies that help identify specific processes driving these effects.

#### CRedit authorship contribution statement

**Thomas Bécu:** Writing – review & editing, Investigation. **Gaël Alvarez:** Writing – review & editing, Resources, Methodology, Funding

acquisition, Conceptualization. **Gaëlle Marliac:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Emma Schwartz:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Olivier Duchene:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Lisa Besson:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Magali Joannin:** Resources, Investigation. **Nicolas Gross:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Stéphane Sauvat:** Resources, Investigation. **Audrey Niboyet:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Sébastien Fontaine:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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#### 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.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2026.110362](https://doi.org/10.1016/j.agee.2026.110362).

#### Data availability

Data will be made available on request.

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