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RESEARCH ARTICLE

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Abstract
Purpose The main goal of this study was to determine if ancient wheat varieties could store more carbon than modern ones in the presence or absence of inputs, due to a likely bigger and deeper root system and a slower mineralization rate.
Methods We conducted a field experiment with four modern and four ancient varieties (released before 1960 and often grown without inputs), with and without chemical inputs (nitrogen, herbicide and fungicide taken as a single factor). Root morphology was assessed by image analysis, potential catabolic activities of fructose, alanine, citric acid by MicroResp™ and overall CO_2 emissions by incubating soil and roots from each modality for 60 days.
Results The breeding type did not affect root traits, substrates respiration nor CO_2 emissions in our environmental conditions. The application of inputs did not affect root traits but influenced the respiration of specific substrates and CO_2 emissions. The most noticeable response was due to the "breeding type x inputs" interaction: inputs increased CO_2 emissions from soil and root tissues of ancient varieties by 19%, whereas no effect was observed for modern varieties.
Conclusion Taken together, our results did not support the hypothesis that ancient varieties could be more performant than modern ones in storing carbon in our experimental conditions. Increased CO_2 emissions by ancient varieties in the presence of inputs showed that ancient and modern varieties differed in their phenotypic plasticity.

Keywords Wheat varieties · Carbon storage · Mineralization rate · Root morphology · Synthetic chemical inputs

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Introduction

Since the industrial revolution, human activities have impacted climate by increasing drastically greenhouse gases (GHGs) fluxes in the atmosphere (Crutzen 2002; IPCC 2019) and among them those containing carbon (CO_2 and CH_4). Anthropogenic carbon emissions could be partially mitigated by favoring the transfer of carbon from the atmosphere to carbon sinks like soils (Friedlingstein et al. 2019; Le Quere et al. 2015). Soils store approximately three times more C stocks than the atmosphere

(2400 vs. 800 GtC) (Jobbagy and Jackson 2000) and can, thus, play a potentially important role in climate change mitigation. In this regard, agricultural soils are of particular interest because they cover 37.4% of the world's land area (FAOSTAT 2016). Because agricultural soils are already managed by farmers, an adaptation of agricultural practices could have important effects on soil carbon stocks (Dignac et al. 2017) with minimal additional costs, as compared with carbon storage strategies developed in natural soils.

Among agricultural practices with a strong leverage effect on carbon sequestration, the choice of the crop is crucial since the plant is responsible for carbon inputs in the soil. The diversity of cultivars provides an important pool of species and genotypes, in which farmers could choose the most adapted to their objectives, including efficient soil carbon storage (Mathew et al. 2020). Wheat varieties are of particular interest since this crop is cultivated on 220 million ha worldwide which represents 4% of agricultural lands (Klein Goldewijk et al. 2017; USDA 2018). Moreover wheat has a high C allocation to the soil (Mathew et al. 2020), which makes it a good candidate for carbon storage. Among the wheat cultivar diversity, farmers can choose between ancient, released before the Green Revolution, or modern varieties, hereafter considered as two "breeding types".

Artificial selection for improved yield in high-input agriculture has led to a decrease in wheat shoot size (Berry et al. 2015). From greenhouse experiments, ancient varieties are also reported to exhibit deeper root systems (Shaposhnikov et al. 2016; Subira et al. 2016) and to show higher root biomass than modern ones (Pour-Aboughadareh et al. 2017; Waines and Ehdaie 2007). Since half of the carbon stored in soil is located below 30 cm (Balesdent et al. 2018), deep root systems represent a credible opportunity to increase carbon storage. Differences in physiology, composition of plant tissues (Gotti et al. 2018; Iannucci et al. 2017) and root architecture (Beyer et al. 2019; Junaidi et al. 2018) between ancient and modern breeding types could also be responsible for reduced root decomposition rates in ancient varieties. Since the exudation profiles between ancient and modern varieties of the same species are supposed to differ (Beyer et al. 2019), microbial communities living in the vicinity of plant roots could also differ in structure and function between them, with potential consequences for root development and dead root mineralization.

Changes in plant genotype due to breeding occurred simultaneously with the increasing use of synthetic chemical inputs (fertilizers, pesticides and herbicides) (Lynch 2007). These inputs are widely applied in the fields since the Green Revolution and are known to directly modify the soil and rhizosphere microbial communities (Nave et al. 2009; Geisseler and Scow 2014). Root morphology is also very sensitive to the addition of nitrogen (N) inputs, with highly different morphological responses, from a reduction to an increase in root system size (Guo et al. 2008; Noguchi et al. 2013; King et al. 1997; Wang et al. 2013). Since modern varieties have been selected in the presence of chemical inputs and are generally grown with them, whereas ancient varieties are grown without, it is possible that these two breeding types respond differently to inputs, especially in terms of carbon allocation and restitution to the soil. It is thus of particular interest to decouple the effect of breeding and input application to assess the relative importance of the genotype (G), the modification of the environment by chemical input application (E), and their interaction (G×E) on carbon allocation to the soil and its mineralization. The effects of breeding and inputs can be described by adopting the formalism of quantitative genetics $P = G + E + G \times E$ (Falconer 1989). In this study, root system biomass and morphology will be considered as phenotypical traits "P". The mineralization rate of this root material, which is under the control of the microbiota recruited by the plant (Lemanceau et al. 2017), can be considered an "extended" phenotype of the plant (Dawkins 1999; de la Fuente Cantó et al. 2020). Functional and structural properties of the microbiota can indeed be considered a phenotypical trait of the host (Walters et al. 2018; Oyserman et al. 2021). These phenotypical traits can be determined by: (i) the plant breeding type "G" (either modern or ancient varieties), (ii) the environment "E" (modified by agricultural practices such as the application of inputs) and (iii) the interaction between crop breeding type and inputs "G×E" (defined as plant phenotypic plasticity). Phenotypic plasticity denotes the ability of a given genotype (here the breeding type) to produce different phenotypes across different environments (Laitinen and Nikoloski 2019); it is often represented as a "norm of reaction", where trait changes of each breeding type are

depicted across environments (Schmalhausen 1949; Stearns 1989).

Most studies trying to compare ancient and modern varieties are made in controlled conditions in the absence of inputs or in nutrient-depleted soils (e.g. Brisson et al. 2019), making it difficult to conclude on the differences between ancient and modern varieties' root systems in the field. Based on a field experiment combining variation in breeding type (ancient and modern varieties) and chemical inputs (either the simultaneous presence of nitrogen, herbicide and fungicide or the absence of the three), we measured, at different depths, root biomass and morphology, and we incubated roots and soils from corresponding plots and soil layers to assess CO_2 emission. CO_2 emissions were considered as a proxy for carbon storage (which integrates the distribution of carbon across different carbon pools), since soil carbon content changes in one year are too weak to be detected in the field, and crop rotation prevents reproducing several years the same experiment exactly at the same place to observe cumulated effects. The purpose of this paper was to test the following hypotheses: (1) the breeding type influences carbon storage either (1a) through increased root biomass and root surface area in ancient varieties, or (1b) a reduced carbon mineralization rate by their associated microbiota; (2) synthetic chemical inputs influence carbon storage either (2a) by reducing root biomass and surface or (2b) by increasing root carbon mineralization; (3) the effect of plant breeding type on (3a) root biomass and morphology or (3b) mineralization rate is dependent on the presence of inputs.

Materials and methods

Field site description

The experiment was carried out in the experimental field of the Institut Agro Dijon (47° 18′ 32" N 5° 04′ 02" E, Dijon, France) from October 2019 to July 2020. The climate is a temperate oceanic climate (Köppen-Geiger classification), characterized by a mean annual temperature of 11.0 °C, mean maximal and minimal temperatures of 15.4 °C and 6.6 °C respectively. Mean annual precipitation calculated on a 30 years duration is 760.5 mm and reference evapotranspiration (ET0) 853.8 mm (Météo France). The study area is dominated by old colluvial and alluvial materials originated from sedimentary calcareous rocks. The main soil type is Calcaric Cambisols (FAO 2014), with a soil texture of organo-mineral horizons dominated by the clay fraction, a soil pH_{H2O} value of 8.23 and soil organic matter (SOM) content of 35.78 g kg^{-1}. This soil was rather thin, with a rock bed at 30 cm depth (for more soil properties, refer to Supplementary material Table S1). No roots were found below this limit. This soil was representative of the soils of the region and in particular those cultivated with wheat, thus a good candidate to assess the performances of wheat varieties in our region. The preceding crop was a field bean (*Vicia faba*) for all the experimental plots.

Plant material and experimental design

A group of eight wheat varieties from two kinds of genotypes, hereafter called "breeding types" was studied: four ancient varieties (A), released before the Green Revolution (before 1960) and four modern varieties (M), released after 1960. Ancient varieties were provided by "Graines de Noé", a non-governmental organization (http://www.graines-de-noe.org/), which promotes the conservation of wheat landraces. Among their 200 varieties, all grown without inputs, we selected some with a local origin, mainly from the Bourgogne Franche-Comté administrative district (Table 1). Modern varieties were selected after the'60 s in high input systems (http://www.fiches.arvalis-infos.fr/) (Table 1).

Seeds were sown on October 29th, 2019 (week 0). Two agronomic treatments were applied for each variety: i) with inputs (w) and ii) without inputs (w/o). In the treatment with inputs, products and doses applied were those commonly used in the Bourgogne Franche-Comté region on winter wheat. Inputs included herbicide (Bofix™, Dow Agro Science, made of fluroxypyr 40 g l^{-1} (3.7%), + clopyralid 20 g l^{-1} (1.8%) + MCPA 200 g l^{-1} (18.4%)), supplied once at 0.3 $l.ha^{-1}$ on April 10th (week 23), fungicide (Bell Star™, Dow Agro Science), applied once at 2.5 $kg.ha^{-1}$ on May 5th (week 26), and fertilizer (CAN 27% Granulé, Dijon Céréales, France) for a total of 150 $kgN.ha^{-1}$, applied as 50 $kgN.ha^{-1}$ in three times, on February the 20th (week 18 after sowing), March 26th (week 25) and May the 30th (week 30). The complete cross-factorial design was made of four

Table 1 Ancient and modern varieties and their date of release

Breeding	Variety	Year of first appearance	Provider
Ancient	Automne Rouge	XIXth century	Graines de Noé
	Barbu du Mâconnais	XIXth–beginning XXth century	Graines de Noé
	Blé de Saône	Before 1960	Graines de Noé
	Alauda	2013 (Probus (1948) X Inntaler (before 1960))	Graines de Noé
Modern	Alixan	2005	Limagrain
	Nemo	2015	Secobra
	Rubisko	2012	RAGT Semences
	Tulip	2011	Saaten Union

modern and four ancient varieties, with or without inputs ($n=16$), replicated in three randomized blocks ($n=48$), on individual plots of 1 m^2 each, separated from each other by 0.8 m. The planting density was 300 seeds per square meter. Wheat grains were manually sown at a depth of 4 cm, and distributed among seven rows (0.15 m apart).

Soil Sampling and Sample preparation

Sampling was carried out on May the 26th 2020, at the tillering stage, by collecting a soil core of 8 cm diameter per plot, just below a plant, after cutting the shoot, down to 30 cm. We divided the soil core into the 0–15 cm and 15–30 cm soil depths. Considering the spatial variability of soil depth, the sampling volume of soil cores was not always the same. To integrate this variability, the data were normalized to 100 cm^3 for analysis. Forty-eight soil samples were retrieved at 0–15 and the same number at 15–30 cm depth. For each sample, we collected rhizosphere and bulk soil. Rhizosphere soil was collected by manual shaking to keep only 1 to 2 mm soil around the roots and brushing of the roots with caution, and, bulk soil was collected by sampling the loose soil not aggregated around the roots and sieving at 2 mm. Roots were also collected. Elutriation (Smucker et al. 1982), a method based on differential sedimentation (Fenwick 1940), was used to retrieve all the root fragments from the soil core: a slight water flux flowing out of a container carried away root pieces while soil particles stay at the bottom of the container due to their higher density (Blouin et al. 2007). Root pieces were recovered and stored in water at 4 °C for further image analysis and incubations. Soil samples (rhizosphere and bulk soils) were stored at -20 °C for 10 months. Root biomass (g in 100 cm^3 of soil) was measured after image analysis and before taking some material for incubation, after drying at 50 °C for two days.

Root system morphology

All roots included in the sample were spread on a tray and scanned at 600 dpi using an Epson GT2000 J151A (Epson America, Inc., Long Beach, USA). Images were analyzed with WinRhizo™ (2013 version, Regent Instruments, Inc., Quebec, Canada). Different morphological traits were measured with this software: length (cm in 100 cm^3 of soil), surface area (cm^2) and root average diameter (mm). We calculated the specific root length (SRL, cm g^{-1}) by dividing root length by root dry biomass.

Incubations

Given that only a small quantity of rhizosphere soil was retrieved, which was just sufficient for the MicroResp technique (see below), the soil used for incubations was bulk soil sieved at 2 mm and air-dried to adjust soil moisture for incubations (Scheu and Parkinson 1994; McGowen et al. 2018). 96 microcosms (eight varieties, two inputs treatments, three replicates per breeding types and inputs treatments and two depths) were set up in 37-ml flasks by placing 3 g of dry weight bulk soil mixed with 15 mg dry weight roots crushed as fine powder (TissueLyser II, Quiagen, Germany, for one minute at 20 oscillations.s^{-1}) retrieved in the same plot and depth as the incubated soil, to preserve, as far as

possible, interactions between specific roots and soil microbial communities. This ratio is commonly used in literature (e.g. Pascault et al. 2013). Soils were watered to 40% of the water holding capacity by adding sterile water. The soil microcosms were then incubated at 20 °C in the dark for 60 days. The gaseous phases of the microcosms were sampled at 1, 6, 12, 20, 32 and 60 days of incubation with a 1 ml air gas syringe and put in 10 ml airtight flasks for measurement of the CO_2 concentration. Microcosms were not aerated during this incubation period since the risk of anaerobic conditions was not significant, the soil volume being very small as compared with the flask volume. CO_2 concentration was determined through gas chromatography using a 990 Micro GC system (Agilent, Santa Clara, USA).

Mineralization of specific substrates

Enzymatic activities of rhizosphere microbial communities were studied using the MicroResp™ technique (Campbell et al. 2003), following the manufacturer's instructions. In brief, the rhizospheric soil of the 48 plots for the two depths was sieved at 2 mm. Soil moistures were calculated on those samples ($14.36 \pm 2.99\%$ for 0–15 cm samples and $13.74 \pm 2.91\%$ for 15–30 cm samples, mean ± s.d.). Deep-well plates filled with these soils were incubated in the dark at 25 °C for 72 h before measurement. In a preliminary test, nine different carbon sources representing amino acids (l-arginine and l-alanine), carbohydrates (d-fructose, d-galactose, d-glucose and l-arabinose) and carboxylic acids (citric acid, l-malic acid and oxalic acid) were tested on a restricted number of samples, leading to the selection of l-alanine, d-fructose and citric acid, which were representative of these three groups of molecules and the most affected by our treatments. Twenty-five microliters of these three different carbon sources were added to the MicroResp™ deep-well plates. Carbon dioxide (CO_2) emission was measured by colorimetry on dye plates, with a spectrophotometer (Infinite M200Pro, Tecan, Männedorf, Suisse) at 570 nm: immediately before placement and after 6-h incubation in the dark at 25 °C. The CO_2 evolution rate was calculated according to the instructions of the manufacturer.

Statistical analysis

Normality and homoscedasticity of the data were tested on the residuals of the models using Shapiro and Bartlett tests respectively, with R default functions. Non-normally distributed data (Root Biomass, Respiration with Fructose and Alanine substrates and total CO_2 release in incubation) were log-transformed.

We tested several models. Since the block had no significant effect, a very limited effect on the residual variance and avoided an inflation of the number of factors (and all their potential interactions) and a consumption of degrees of freedom, this factor was removed from the final model. All variables were thus analyzed with the following three-way ANOVA model:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + E_{ijkm}$$

Y_{ijkm} is the studied parameter, of breeding type i, under inputs treatment j, at soil depth k. μ is the general effect. α_i is the effect of the breeding type (qualitative: ancient, modern), β_j is the effect of the inputs treatment (qualitative: with, without). γ_k is the effect the soil depth (qualitative: 0–15 cm, 15–30 cm). $(\alpha\beta)_{ij}$is the interaction effects between the breeding type and the inputs treatment; $(\alpha\gamma)_{ik}$ is the interaction effects between the breeding type and the soil depth; $(\beta\gamma)_{jk}$ is the interaction effects between the inputs treatment and the soil depth and $(\alpha\beta\gamma)_{ijk}$ is the interaction effects between the breeding type, the inputs treatment and the soil depth. E_{ijkm} is the residual error. These analyses were followed by a post-hoc Tukey'Honest Significant Difference test ($p < 0.05$, package 'agricolae', (De Mendiburu 2017)). To analyze the data of CO_2 emissions for each date of measurement along the incubation time (non-independent data), the factor Date was included in the model as a simple effect (without interaction due to the number of degrees of freedom) (Table S2). We also analyzed the CO_2 cumulated at the end of the incubation period (60 days) (Table 3, Fig. 3).

We represented the results of these ANOVAs by plotting the dependent variable in response to the two environmental modalities (without "w/o" and with "w" inputs) and in conjunction with phenotype's responses related to a given breeding type. This representation is the one used to represent phenotypic

plasticity and more generally interaction between a genotype and its environment (Oyserman et al. 2021).

Analyses of root traits, MicroResp™ and incubations data were performed with the RStudio software (RStudio Team 2020).

Results

Effects of breeding type and inputs on roots biomass and morphology

Root biomass and morphology present similar responses to the different factors (Table 2). Depth had a significant effect on all root traits (root biomass, root length, root average diameter and root surface area), explaining between 53.2 and 81.3% of the variance, for root average diameter and root biomass respectively (Table 2, Fig. 1a, b, c, and d versus 1e, 1f, 1 g, 1 h). Breeding type (ancient *vs* modern varieties) had no significant effect on root biomass, root average diameter and root surface area (Table 2). The breeding type had a marginal effect ($p=0.084$) on root length (explaining 1.38% of the total variance) (Table 2). The breeding type had no significant effect on the specific root length (length of root per gram of dry root, $p=0.29$, data not shown). We also found no effect of inputs on roots traits (Table 2). However, some trends were observed. At 0–15 cm, ancient varieties in the absence of inputs had the highest observed root length and root surface area, but the difference with other treatments was not significant after post-hoc correction for multiple comparisons (Fig. 1c and d).

Effects of breeding type and inputs on C mineralization

Total CO_2 release was impacted by the depth, the presence/absence of inputs and the breeding type (Table S2, Fig. 2). The interaction between breeding type and inputs also had a significant effect on total CO_2 release, as well as the interaction between breeding type and depth (Table S2). To study the effect of breeding type and inputs on C mineralization independently of the time and depth, we studied the quantity of CO_2 cumulated at day 60 (Table 3). After 60 days of incubation, the breeding type did not affect the quantity of CO_2 released (Table 3). For ancient varieties, the quantity of CO_2 emitted was 19% higher in the presence of inputs ($p=0.0342$), but for modern varieties, inputs had no significant effect ($p=0.999$) (Fig. 3).

Effects of breeding type and inputs on the degradation of selected substrates

Breeding type (ancient *vs* modern varieties) had no effect on CO_2 emission for the alanine, a significant effect for the citric acid (explaining 2.39% of the total variance, $p=0.050$) and a significant effect for the fructose (1.21%, $p<0.019$) (Table 4, Fig. 4). The presence/absence of inputs had a significant effect on

Table 2 Analysis of variance for morphological root traits

	Root biomass (g in 100 cm^3 of soil)		Root average diameter (mm)		Root Length (cm in 100 cm3 of soil)		Root surface area (cm^2)	
	% Sum Sq	F value	% Sum Sq	F value	% Sum Sq	F value	% Sum Sq	F value
Breeding type	0.11	0.56	0.74	1.51	1.38	3.05	0.040	0.11
Inputs	0.044	0.22	0.37	0.75	0.23	0.52	0.012	0.034
Depth	81.3	398.6 ***	53.2	108.2 ***	57.1	126.4 ***	68.5	198.4 ***
Breeding type:Inputs	0.13	0.64	0.28	0.56	0.30	0.68	0.32	0.92
Breeding type:Depth	0.25	1.24	0.68	1.52	0.16	0.37	0.099	0.28
Inputs:Depth	0.13	0.64	0.62	1.27	0.98	2.17	0.29	0.86
Breeding type:Inputs:Depth	0.022	0.10	0.87	1.76	0.002	0.004	0.32	0.92
Residuals	17.9		43.3		39.8		30.4	
% of variance explained	82.0		56.7		60.2		69.6	

Data were log-transformed to respect normality and homoscedasticity for root biomass. Percentages of sum square and F values are given, with asterisks indicating the significance of effects. $P<0.10$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

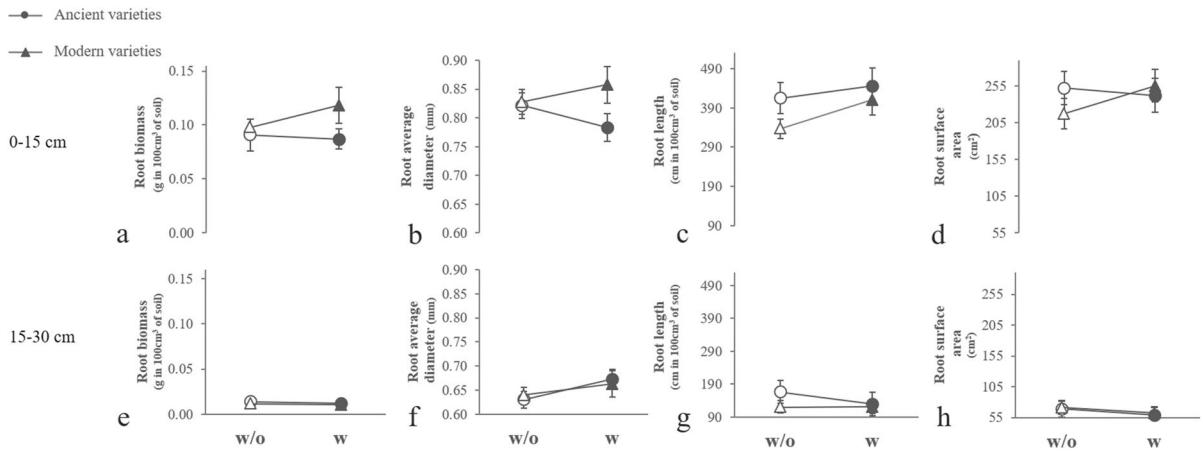

Fig. 1 Morphological root traits presented as reaction norms of ancient and modern wheat breeding types to the modification of the environment by inputs (w/o: without inputs, (white marks; w: with inputs, black marks). Panels are respectively showing: the root biomass at 0–15 cm (**a**) and 15–30 cm (**e**), the root average diameter at 0–15 cm (**b**) and 15–30 cm (**f**), the root length at 0–15 cm (**c**) and 15–30 cm (**g**) and the root surface area at 0–15 cm (**d**) and 15–30 cm (**h**) mean ± se. $n = 12$ for each treatment

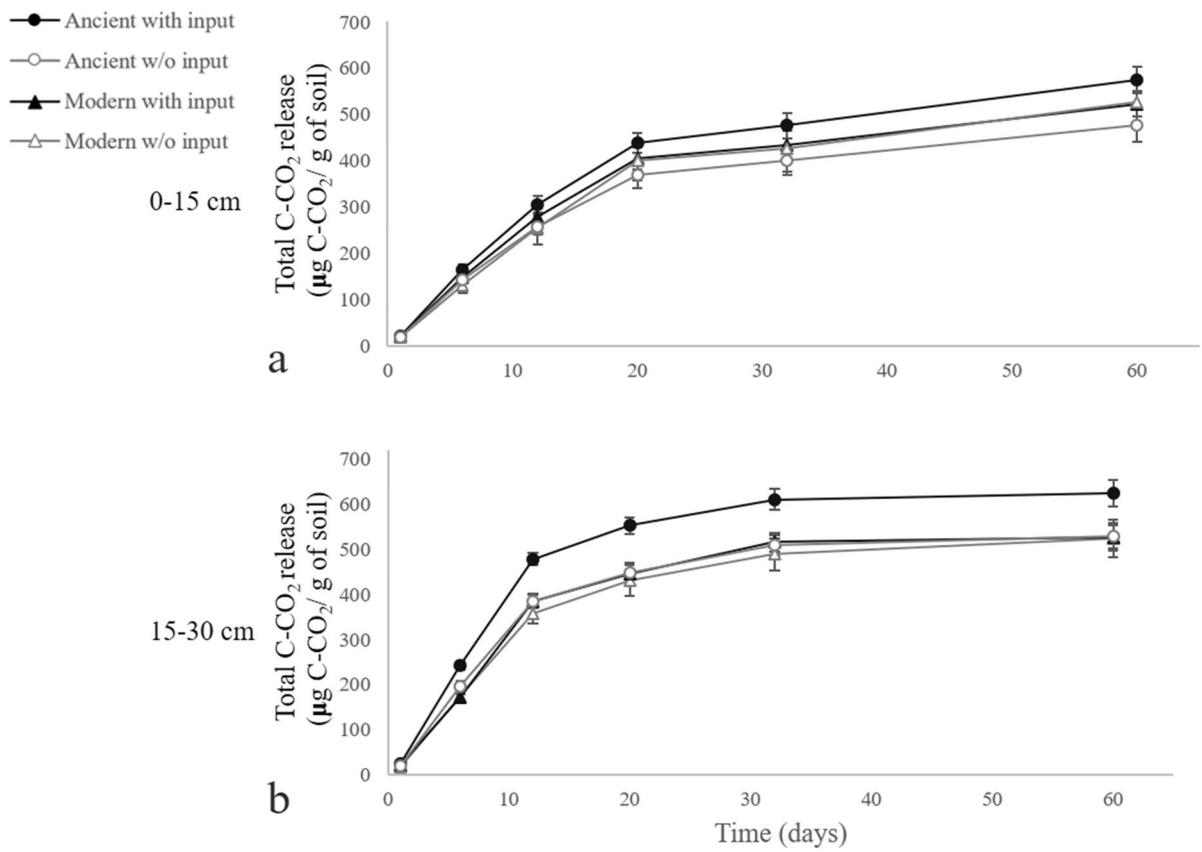

Fig. 2 Total CO_2 accumulated during the 60 days of incubation for microcosms amended with wheat root residues from modern (triangles) and ancient (circles) varieties in soil from 0–15 cm depth (**a**) and 15–30 cm depth (**b**). $\alpha = 0.05$. mean ± se. $n = 11$–12 for each treatment

Table 3 Analysis of variance for CO_2 released after 60 days of incubation

Total CO_2 released after 60 days (µg C-CO_2/ g)			
	% Sum Sq	F value	*P* value
Breeding type	1.02	1.01	0.32
Inputs	3.78	3.73	0.056
Breeding type:Inputs	3.96	3.91	0.051
Residuals	91.2		
% explained by factors	8.7		

Percentages of sum square, F values et *P* values are given, with asterisks indicating the significance of effects. $P<0.10$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

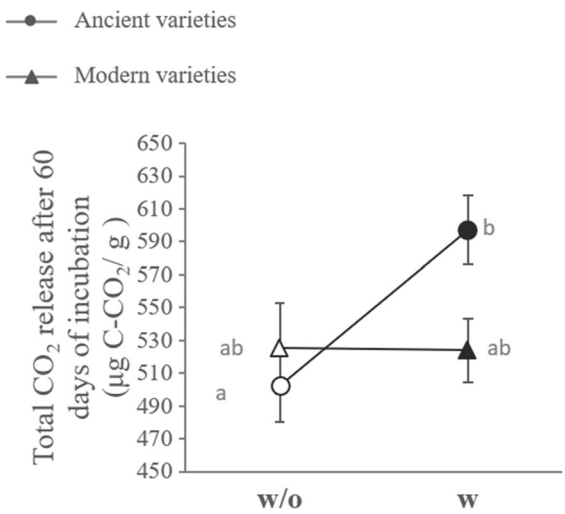

Fig. 3 Total CO_2 released after 60 days of incubation for microcosms amended with wheat root residues from modern (triangles) and ancient (circles) varieties in soil without input (white marks) or with inputs (black marks). Significant differences are represented by different letters. $\alpha=0.05$. mean ± se. $n=11$–12 for each treatment

CO_2 emission for fructose (5.83%, $p=0.015$), alanine (5.49%, $p<0.001$) and citric acid (17.1% variance, $p<0.001$) (Table 4, Fig. 4). At 0–15 cm, considering both ancient and modern varieties together, the addition of inputs led to increased respiration for fructose (Fig. 4a). At 15–30 cm, the presence of inputs was responsible for a significant decrease of respiration with fructose (opposite to observations at 0–15 cm) and alanine, and a significant increase of respiration for citric acid (Fig. 4d, e, and f). Depth had a significant effect for all substrates, explaining 19.3, 59.7 and 61.4% of the variance, for citric acid, fructose and alanine respectively (Table 4, Fig. 4).

Discussion

Ancient varieties were selected and were grown mostly without synthetic chemical inputs in organic farming systems, whereas modern ones were selected and are usually grown with chemical inputs. It is not common to have a fully crossed factorial design allowing to assess the relative importance of individual factors of breeding type and inputs (respectively G and E) and their interaction (G×E) in root morphology and mineralization rate, which are relevant to identify plants able to store more carbon in the soil. Our results are a first attempt to quantify these environmental and genotypic effects independently, in the field. It should however be stressed that if root morphology parameters were indeed obtained directly from the field, the CO_2 emission were measured in the laboratory, since soil carbon content changes in one year are too weak to be detected in the field, and crop rotation prevents reproducing several years the same experiment exactly at the same place to observe cumulated effects. Performing this measure in the laboratory allows to control the amount of incubated root material, but implies that the soil is destructured which could affect the mineralization rate (Salome et al. 2010).

Effects of breeding on root system and mineralization rate

The breeding type did not affect the root biomass and morphology when considered independently of the presence/absence of inputs and depth (Table 2). This result did not support our first hypothesis (1a) regarding a higher root biomass production or a higher external surface in ancient varieties. Due to the introduction of dwarfism genes in modern wheat varieties, responsible for a lower height, a decrease of root system size could have been expected because of the functional equilibrium between root and shoot functions, which states that the root mass and rate of absorption are proportional to the leaf mass and rate of photosynthesis (Davidson 1969; Wilson 1988; Feller et al. 2015). A negative impact of the dwarfism genes on root morphology has already been observed

Table 4 Analysis of variance for respiration rates obtained from MicroResp™

	Fructose respiration (µg/g/h C-CO_2)		Alanine respiration (µg/g/h C-CO_2)		Citric Acid respiration (µg/g/h C-CO_2)	
	% Sum Sq	F value	% Sum Sq	F value	% Sum Sq	F value
Breeding type	1.21	5.67 *	0.52	1.55	2.39	3.93
Inputs	5.83	27.3 ***	5.49	16.4 ***	17.1	28.1 ***
Depth	59.7	279.7 ***	61.4	183.5 ***	19.3	31.8 ***
Breeding type:Inputs	0.013	0.060	0.11	0.32	0.002	0.003
Breeding type:Depth	0.021	0.11	0.007	0.021	0.005	0.008
Inputs:Depth	14.4	67.6 ***	2.76	8.26 ***	7.42	12.2 ***
Breeding type:Inputs:Depth	0.11	0.52	0.29	0.88	0.31	0.51
Residuals	18.8		29.4		53.5	
% of variance explained	81.2		70.6		46.5	

Data were log-transformed to respect normality and homoscedasticity for fructose and alanine. Percentages of sum square and F values are given, with asterisks indicating the significance of effects. $P<0.10$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

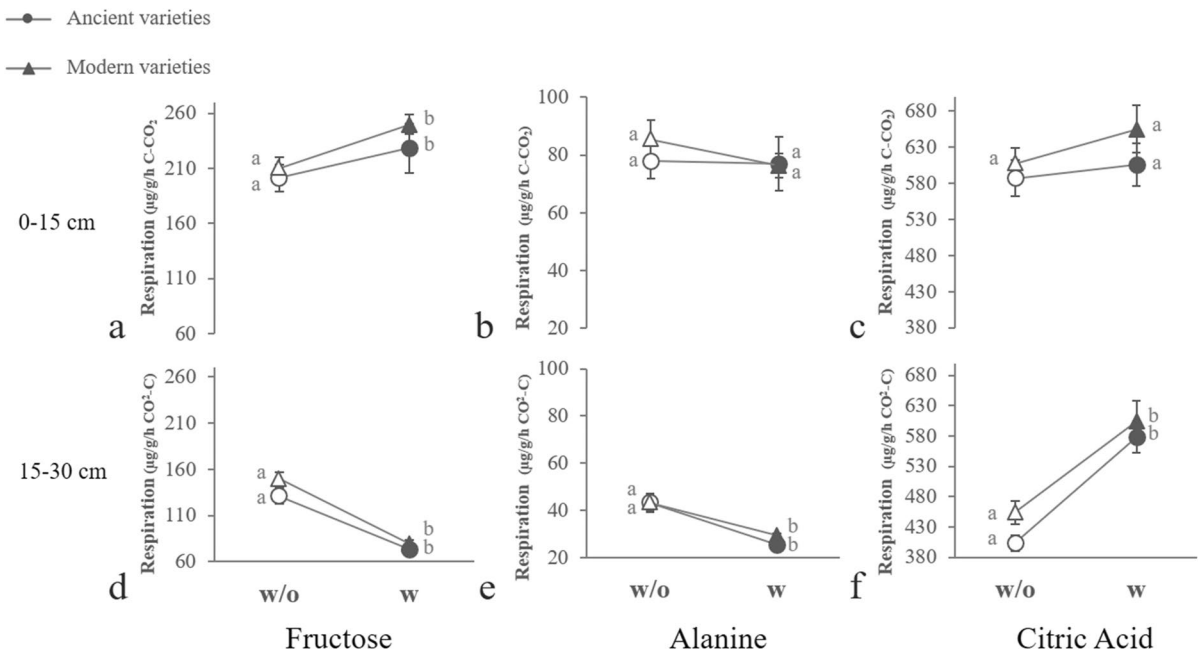

Fig. 4 Respiration data from MicroResp™ plates, presented as reaction norms of ancient and modern wheat breeding types to the modification of the environment by inputs (w/o: without inputs, white marks; w: with inputs, black marks). Panels are respectively showing the respiration in presence of different substrates: Fructose at 0–15 cm (**a**) and 15–30 cm (**d**), Alanine at 0–15 cm (**b**) and 15–30 cm (**e**) and Citric Acid at 0–15 cm (**c**) and 15–30 cm (**f**). Significant differences are represented by different letters. $\alpha=0.05$. mean ± se. $n=12$ for each treatment

(Subira et al. 2016; Pour-Aboughadareh et al. 2017). In the study of Laperche et al. (2006), the Rht1 gene has a negative impact on primary and lateral root length and leads to a decrease in root biomass for modern varieties. This was not observed in our study. It may be due to differences in experimental conditions: (i) some studies compare modern varieties with wild relatives, and not ancient varieties (Pour-Aboughadareh et al. 2017). Our results thus suggest that domestication from wild ancestors to cultivated

varieties could have had a different impact, especially on root traits, that the recent selection effort from ancient to modern varieties; (ii) others were conducted in greenhouses, in tubes filled with a soil/sand mixture (Subira et al. 2016), so in experimental conditions relatively far from the field. Another explanation could be that the thin soil in our site (30 cm depth) was not deep enough for ancient breeding types to exhibit their stronger root development. Implementing experiments in diversified pedological contexts could help in identifying the soil characteristics in which ancient varieties exhibit bigger and deeper root systems.

A key process for carbon storage is organic matter mineralization. At 60 days of incubation of root material with its surrounding soil, we observed no difference in CO_2 emissions between ancient varieties and modern ones (Table 3). Therefore, this result invalidated our first hypothesis (1b): there was no reduced carbon mineralization rate with ancient varieties. Shaposhnikov et al. (2016) showed that the total amount of sugars (mostly fructose, glucose and maltose) exuded by modern varieties was three to five times higher as compared to ancient varieties. If there were more exudates produced by the modern varieties, they could have been metabolized before the incubations. However, it is difficult to compare between experiments since the identity of the varieties differs and that the number of representatives of modern and ancient varieties is low.

When focusing on the catabolic activities of specific substrates in the rhizospheric soil with MicroResp™, we found the highest respiration rate for modern varieties (+8.72% for fructose, Fig. 4 and Table 4). Some differences between the two methods and our protocol could also be important. MicroResp is measuring the catabolic activity on added substrates (i.e. a potential for each substrate), while incubation is measuring basal respiration of soil and roots. The incubation time differs (6 h and 60 days, for MicroResp™ and incubations respectively) and microorganisms involved in the first steps of C mineralization (r-strategists feeding on easily degraded substrates) are not the same as the ones implied in later steps (K-strategist feeding on more complex substrates) (Fontaine et al. 2003; Cayuela et al. 2009). Regarding our protocol, we used rhizosphere soil for MicroResp and bulk soil for incubations. To conclude, we found a significant effect of the breeding type on CO_2 emissions only with rhizospheric soil on the short term (6 h). Since this was not observed in 60 days incubations with root material in bulk, it stresses on the importance to precise the nature of the soil used in incubation in future studies, or to test both bulk and rhizospheric soils to conclude on the effect of the breeding type.

Effects of inputs on root system and mineralization rate

The presence/absence of inputs had no detected impact on root biomass and some morphology parameters such as length, surface area and average diameter. However, a more detailed analysis of other root traits, such as lateral roots-related parameters (length or branching frequency), could give different results and conclusion. We thus rejected the hypothesis that synthetic chemical inputs reduced root biomass and surface (2a) with consequences on carbon storage. This surprising result could first be explained by the fact that the preceding crop was a legume (*Vicia faba*), which was likely responsible for an already high N level in the soil, even for plots without N fertilizer (Table S1). In the literature, some studies show a decrease in root growth with the addition of N (e.g. Wang et al. 2013), interpreted as an adaptation based on the cost/benefit ratio. However, in a meta-analysis, Xia and Wan (2008) showed that the addition of N stimulated the growth of roots, with an increase of 15.6% of the root biomass. This apparent contradiction can be explained by the fact that when nitrogen is supplied externally, the total energy budget of the plant is changing, which can increase shoot and root biomass, or induce a different partitioning of resources between shoots and roots. In our specific case, the effects due to the cost/benefit ratio and the increased energy budget could cancel each other out. In addition, the availability of other soil nutrients and the stoichiometry of plant tissues (Sterner and Elser 2002), i.e. the ratio between the different elements such as C, N, P…, could explain this discrepancy. Moreover, climate, particularly temperature, and plant functional types, i.e. plant with common features associated with their function, are a strong determinant of root trait variation (Freschet et al. 2017).

The presence/absence of inputs had a significant effect on C mineralization (Table S2, Fig. 2). At the

end of incubation (Table 3, Fig. 3), 9% more CO_2 had been emitted by soils that had received inputs (independently of G, but see the section below about phenotypic plasticity) as compared with soils without inputs. We thus validated hypothesis 2b that the addition of inputs increases CO_2 emissions from the soil. Some previous studies have shown that the addition of N to soils can have variable effects on soil microbial respiration, including increase, decrease, or unchanged rates of mineralization (Bowden et al. 2004; Traoré et al. 2007), but despite possible negative priming effects (Kuzyakov et al. 2000), available organic carbon, nitrogen or phosphorus addition generally increase microbial activity (Teklay et al. 2006).

When considered independently of the breeding type and depth, inputs were responsible for an effect on the respiration of specific substrates: a decrease of 9% and 17% for fructose and alanine, respectively; and an increase of 19% for citric acid. For citric acid, the addition of inputs may have suppressed an N limitation and allowed microorganisms to decompose recalcitrant C. For fructose and alanine, the decrease of respiration observed may be due to a negative priming effect, described by Kuzyakov et al. (2000): the addition of N fertilizer may have led to a preferred uptake of C-rich substrates by microorganisms. The substrates chosen for this study are classified as being potentially responsible for either a positive or a negative priming effect (Dalenberg and Jager 1989), but the mechanisms responsible for a change in the sign of the effect are not yet understood. Since we added pesticides and fertilizer simultaneously, complex effects likely emerge. The decrease of respiration for fructose and alanine may be due to the presence of fungicide. Fungal species are known to be the primary producers of β-glucosidase in soils (Plaza et al. 2004). β-glucosidase catalyzes the hydrolysis of cellobiose and thus plays a major role in the initial phases of decomposition of organic C compounds. Even if fungicides are supposed to target specific fungal pathogens, the impact on fungal communities has been already observed (Esmaeili Taheri et al. 2015). Fungicide probably affected fungi resulting in a decrease of β-glucosidase activity and thus a decrease of C mineralization. It may have compensated the effect of N fertilizer for modern varieties, supposed to increase C mineralization.

Effects of phenotypic plasticity on root system and mineralization rate

Phenotypic plasticity – "G×E" – describes the ability of a genotype (here a breeding type) to produce different phenotypes in response to variation in environmental conditions (Gause 1947; Bradshaw 1965). Our experimental design allowed us to assess whether the CO_2 released was affected by the interaction between the breeding type and the presence of inputs.

There was no effect of the interaction between the breeding type and the presence of inputs on root biomass and studied root traits (Table 2) nor on the degradation of selected substrates (Table 4), but there was an effect of this interaction on overall CO_2 release (Table 3). For ancient varieties, total C release after 60 days of incubation increased by 19% with the addition of inputs, whereas for modern varieties, the presence of inputs did not affect total C release. We thus validated hypothesis 3b, that the effect of plant breeding type on mineralization rate is dependent on the presence of inputs, not the 3a, since root biomass and morphology were not affected by the G×E interaction. Since it is unlikely that root tissue composition differs strongly between ancient and modern wheat varieties (Gotti et al. 2018), this effect may be due to an increased dominance of specific microbial species in the rhizosphere of ancient varieties in the presence of inputs (Jacquiod et al. 2022). We propose that these specific taxa could exhibit catabolic activities particularly well suited to degrade roots of ancient varieties in the presence of N.

To conclude, there was no effect of the breeding type on root traits, biomass, substrate respiration and CO_2 emissions. Inputs were significantly changing substrate respiration and CO_2 emissions. An originality of our study was to demonstrate that inputs increased strongly the CO_2 emissions from the root mineralization of ancient varieties, whereas they had no effects with modern ones. An interesting perspective would be to identify the catabolic activities of soil microbiota associated with ancient varieties that are responsible for these increased CO_2 emissions.

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Author contributions MB, LR and SF conceived the research. EP and LR carried out the experiment. LR, EP, FB and MB retrieved the soil and roots in the field. LR and MB performed root morphology measures, LR and SF the MicroResp measures, CH and LR the gas measures. LR performed statistical analyses and edited the figures and tables with the help of MB and SF. LR wrote the paper with significant inputs from MB and SF. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare they have no conflict of interest.

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