Identification of barley genetic regions influencing plant-microbe interactions and carbon cycling in soil

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ORIGINAL ARTICLE

Identification of barley genetic regions influencing plant–microbe interactions and carbon cycling in soil

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Abstract

Purpose Rhizodeposition shapes soil microbial communities that perform important processes such as soil C mineralization, but we have limited understanding of the plant genetic regions influencing soil microbes. Here, barley chromosome regions affecting soil microbial biomass-C (MBC), dissolved organic-C (DOC) and root biomass were characterised.

Methods A quantitative trait loci analysis approach was applied to identify barley chromosome regions affecting soil MBC, soil DOC and root biomass. This was done using barley Recombinant Chromosome Substitution Lines (RCSLs) developed with a wild accession (Caesarea 26-24) as a donor parent and an elite cultivar (Harrington) as recipient parent.

Results Significant differences in root-derived MBC and DOC and root biomass among these RCSLs were observed. Analysis of variance using single nucleotide polymorphisms genotype classes revealed 16 chromosome regions influencing root-derived MBC and DOC. Of these chromosome regions, five on chromosomes 2H, 3H and 7H were highly significant and two on chromosome 3H influenced both root-derived MBC and DOC. Potential candidate genes influencing root-derived MBC and DOC concentrations in soil were identified.

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Conclusion The present findings provide new insights into the barley genetic influence on soil microbial communities. Further work to verify these barley chromosome regions and candidate genes could promote marker assisted selection and breeding of barley varieties that are able to more effectively shape soil microbes and soil processes via rhizodeposition, supporting sustainable crop production systems.

Keywords Barley (*Hordeum vulgare*) · Crop breeding · Plant–microbe interactions · Quantitative trait loci (QTL) mapping · Soil microbial biomass carbon · Sustainable agriculture

Introduction

Soil microbes mediate carbon (C) and organic matter cycling in soil, contributing a vital role for the regulation of CO_2 emissions from soil (Prentice et al. 2001; Li et al. 2013) and nutrient release from soil organic matter (SOM) (Fontaine et al. 2011; Dijkstra et al. 2013; Alegria Terrazas et al. 2016). The soil microbial communities and their interactions with plants are impacted by the release of a range of compounds from living roots through root exudation, sloughed cells, mucilage and so on, collectively defined as rhizodeposition (Jones et al. 2004). These rhizodeposit compounds, in particular root exudates, are utilized by microbes as C sources to derive energy for their activity (Paterson 2003; Cheng and Kuzyakov 2005), with this resulting in the decrease or increase of SOM decomposition (Jenkinson et al. 1985; Kuzyakov et al. 2000; Yin et al. 2019). Indeed, it is known that the growth of plants can alter (via rhizodeposition) this microbially mediated SOM decomposition to varying extents (e.g. Cheng et al. 2003; Mwafulirwa et al. 2016, 2021), with increases of up to 380% relative to unplanted soil reported by Cheng et al. (2003).

There is growing evidence that plant influences on soil microbial communities, and the functions they undertake, vary not only between plant species but also between individual genotypes within a single plant species. For example, studies by Aira et al. (2010), Bouffaud et al. (2012), Peiffer et al. (2013) and Walters et al. (2018) suggest that rhizosphere microbial community composition under maize is related to plant genotype. In barley, our previous findings (Mwafulirwa et al. 2016, 2017) and those of Pausch et al. (2016) are indicative that soil microbial activity and, in turn, the decomposition of SOM are also impacted by plant genotype. However, there is a lack of knowledge about the plant genetic regions and plant genes influencing these plant–microbe interactions. Identifying the plant genes influencing these interactions could, in particular, underpin the breeding of crop varieties to control microbially mediated soil processes (such as C mineralization). Hence, better understanding of the plant genes influencing (via rhizodeposition) soil microbes and SOM dynamics could help inform crop breeding to support sustainable agricultural production.

A major limitation for crop breeding to control soil processes, and in turn agricultural and/or environmental sustainability, is the current loss of beneficial plant traits associated with soil microbial interactions in the elite gene pool. This is because the development of modern crop cultivars through selection for yield and other beneficial crop plant traits (such as tolerance to biotic and abiotic stresses), usually under intensive chemical fertilizer applications to soil, have resulted in the loss of the plant genetic variation influencing plant-soil interactions (Tanksley and McCouch 1997; Wissuwa et al. 2009). To overcome this problem, others have proposed the use of wild relatives of crop species as donors of exotic germplasm to improve elite varieties. For example, Matus et al. (2003) developed a population of Recombinant Chromosome Substitution Lines (RCSLs) using wild barley *Hordeum vulgare* subsp. *spontaneum* (Caesarea 26-24) as a donor and *Hordeum vulgare* subsp. *vulgare* (Harrington, a North American malting cultivar) as the recurrent parent. Importantly, Caesarea 26-24 is adapted to specific soil conditions, in the view that it was collected in a dry and saline environment in Israel (Matus et al. 2003). This suggests that this accession could provide vital genes for regulating plant-soil interactions, especially for stress adaptation/tolerance promoting sustainable production. Our previous work investigating the impacts of plant intraspecific variation on SOM decomposition using a small number of these RCSLs showed that plant genotype influenced microbial activity and soil functioning (Mwafulirwa et al. 2016). The genetic composition of these RCSLs (Close et al. 2009; Comadran et al. 2012; de la Fuente Cantó et al. 2018) and

a minimum set representing the entire genome of the wild donor parent (de la Fuente Cantó et al. 2018) have been previously determined using mapped single nucleotide polymorphisms (SNPs). The usefulness of minimum sets of introgression lines representing the entire genome of the donor parent for the detection of quantitative trait loci (QTL) effects and identification of novel exotic alleles is demonstrated by several QTL studies in crop plants, facilitating rapid screening of genetic variation for traits requiring detailed or complex phenotypic evaluations (Schmalenbach et al. 2009; de la Fuente Cantó et al. 2018). For example, Prudent et al. (2009) and Tripodi et al. (2020) used 20 and 39 tomato introgression lines, respectively. De la Fuente Cantó et al. (2018) used 28 barley RCSLs to evaluate allelic variation for important agronomic traits.

Here we used these 28 barley RCSLs, that together represent the whole genome of the wild donor Caesarea 26-24 in the genetic background of the elite variety Harrington, to assess the variation in selected root and soil microbe related traits, i.e. root biomass, dissolved organic-C (DOC) and microbial biomass-C (MBC). Previous studies (Blagodatskaya et al. 2009; Tian et al. 2012; Mwafulirwa et al. 2016) showed that soil DOC and MBC are strongly related to soil respiration rates, and thus to soil functioning. The specific objectives of the present study were to (i) determine the range of variation of the impacts of the barley RCSLs on DOC in soil solution and soil MBC using a ^{13}C-CO_2 isotopic labelling approach, and (ii) apply a QTL analysis approach with these RCSLs to identify the barley chromosome regions and potential candidate genes influencing soil DOC and MBC.

Materials and methods

Soil type

The soil was sampled from a conventionally managed field at Balruddery farm (56.4837° N, 3.1314° W) near Dundee, Scotland, from a depth of 0–10 cm and was sieved to < 6 mm onsite before storing at 4 °C for one week. The soil was a sandy loam of Balrownie Series, Balrownie Association, as identified by Bell et al. (2014, unpublished), and had an organic matter content of 5.8% (muffle furnace, 450 °C, 24 h), pH of 6.0 (H_2O) and water content (w/w) of 16.9%. The field was planted with barley that was at vegetative (i.e. stem elongation) stage during soil sampling.

Plant materials and genotyping

Twenty-eight barley RCSLs developed using a wild donor (Caesarea 26-24, from a dry and saline region in Israel) and an elite cultivar (Harrington, a North American malting variety) as recipient parent via an advanced backcrossing strategy were used (Matus et al. 2003). These lines represent the minimum number covering the entire wild donor genome, and each line carries a small introgression of the wild barley genome in the predominantly elite background (Generation Challenge Program, unpublished). These lines were chosen because (i) they represent a unique source of genetic diversity to study plant-soil interactions (since they were derived using a wild barley accession adapted to unique soil and environmental conditions), and (ii) selected lines from this population showed differences in rhizodeposition-derived C and the respective impacts on MBC, DOC and SOM mineralization, as observed in our earlier studies (Mwafulirwa et al. 2016, 2017). The genetic architecture of each line was determined from earlier work (de la Fuente Cantó et al. 2018), using the barley iSelect SNP chip (Comadran et al. 2012). Both parent genotypes (Caesarea 26-24 and Harrington) were used for phenotype evaluation, while only the elite parent Harrington was used as a control for genetic analysis.

Experimental setup and ^{13}C labelling

Soil was packed in 93 pots (22.5 cm × 5.5 cm) to a bulk density of 1 g cm^{-3} and adjusted to 60% water holding capacity (the soil packing volume in each pot was 20.0 cm × 5.5 cm). After one week of soil stabilization in pots, soil solution samplers (Rhizon™ SMS, Rhizosphere Research Products, Wageningen, Netherlands) were inserted to 10 cm depth for DOC measurements. The system was left to stabilize to conditions used in the experiment for a further week before planting.

The barley plants were grown over 39 days without fertilizer addition to soil. Each pot was planted with one of the 30 genotypes (28 RCSLs plus parental

genotypes), with one plant per pot, and fallow pots with soil only were included as a control treatment. These were replicated ($n=3$) in a randomized complete block design under controlled environment conditions within a plant growth chamber (Conviron CG90; Winnipeg, Canada) at 22 °C and 70% relative humidity. Soil water content was maintained by adding deionized water on a mass basis twice a week. A 12 h daily photoperiod was set with 512 µmol m^{-2} s^{-1} PAR within the chamber. Watering was done during the dark period to avoid disruption to the labelling atmosphere.

Labelling plants with ^{13}C–CO_2 started at the seedling growth stage, one week after sowing seeds. This was achieved by passing a continuous flow of ^{13}C-enriched CO_2 (20 atom% ^{13}C) through the plant growth chamber continuously over the experiment period, which was derived by blending CO_2-free air routed via pressure swing adsorption CO_2 scrubber unit (Parker Balston, Haverhill, USA) with 99 atom% excess ^{13}C–CO_2 (Sercon Ltd., Cheshire, UK) and CO_2 from a standard CO_2 cylinder (BOC, Worsley, UK) via Brooks thermal mass flow controllers (Flotech Solutions Ltd., Stockport, UK).

Phenotyping

Measurement of DOC was done at 30d and 39d (harvest point), MBC was measured at 39d, and plant biomass (separated into root and shoot biomass) was quantified at 39d. For DOC sampling, vacuum sealed 10 mL bottles were connected to Rhizon soil solution samplers via needle ends and left overnight. The vacuum allowed the soil solution to be drawn into the bottle. Bottles were detached and the collected solutions were kept frozen until they could be analysed for total organic C (TOC) concentration (Shimadzu TOC analyser, Japan) and ^{13}C-enrichment of this TOC. Soil solution TOC was assumed to represent total DOC. The ^{13}C-enrichment of DOC was determined using a method described by Garcia-Pausas and Paterson (2011). In brief, the frozen soil solutions were defrosted and sufficient solutions to contain a minimum of 20 µg C and a maximum of 60 µg C per sample, as established from previous TOC analysis of the solutions, were dispensed into muffle-furnanced 12 mL Exetainer vials (Labco Ltd., High Wycombe, UK). Maximum volume in the Exetainers was 4 mL, and deionized water was added to reach the 4 mL volume if a lesser volume was taken. An aliquot of 100 µL of 1.3 M phosphoric acid was added to each sample to remove dissolved inorganic C from the solution. The soil solutions were left with caps off for one hour, following which the vials were capped and flushed with CO_2-free air on the gas bench. To evolve organic C as CO_2, an aliquot of 100 µL 1.05 M sodium persulphate was injected into each solution sample through the rubber septum of the vial-cap. The samples were then heated on a dry block at 90 °C for 30 min to release CO_2. The CO_2 released from the soil solution was then transferred to pre-evacuated N_2 flush-filled Exetainer vials using a syringe with a flow control valve. The ^{13}C-enrichment of the CO_2 sample was determined on a Delta^{PLUS} Advantage isotope ratio mass spectrometer via an interfaced Gasbench II unit (both Thermo Finnigan, Bremen, Germany). The measured ^{13}C-enrichment (atom% ^{13}C) of soil solutions were used to separate root-derived DOC (DOC_{plant}) and SOM-derived DOC (DOC_{soil}) proportions of total DOC (DOC_{total}) following Eqs. 1 and 2 (Garcia-Pausas and Paterson 2011).

$$DOC_{plant} = DOC_{total}\left(atom\%^{13}C_{control} - atom\%^{13}C_{total}\right) / \left(atom\%^{13}C_{control} - atom\%^{13}C_{plant}\right) \quad (1)$$

$$DOC_{soil} = DOC_{total} - DOC_{plant} \quad (2)$$

where atom% $^{13}C_{control}$ is the mean atom% ^{13}C of DOC measured in the unlabelled fallow control treatments and atom% $^{13}C_{total}$ is the measured atom% ^{13}C value of sample total DOC. Atom% $^{13}C_{plant}$ is the atom% ^{13}C value of the plant tissue (described below).

At harvest, soils were re-wetted to initial moisture level by adding deionized water. Plant shoots were harvested by cutting at the soil surface. Roots were carefully removed from soil (shaking off most of the adhering soil) and put in separate containers then washed with deionized water. The harvested plant shoot and root fractions were taken for freeze-drying. The soil was harvested as one fraction, considering that the soil was densely colonised by roots. The fresh harvested soil was thoroughly mixed by hand and immediately stored at 4 °C for subsequent analyses of soil MBC. The dry weights of root and shoot fractions were used to quantify root and shoot biomass, and total plant biomass was calculated as root

plus shoot biomass. Dried root and shoot samples were ball-milled (Retsch Ball Mill, model MM2000) and analysed for ^{13}C-enrichment on a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany).

The fresh soil samples were used to determine the soil MBC concentration, within 24 h following harvest. Two sub samples of the fresh harvested soil were used for MBC analysis. MBC was determined by the chloroform fumigation-extraction method (Vance et al. 1987), where fresh fumigated and non-fumigated soil samples (equivalent 12.5 g dry soil) were extracted with 50 mL of 0.5 M K_2SO_4 solution. Organic C of the extracts was analysed on a TOC Analyser 700 (Corporation College Station, TX). MBC was calculated as the difference between organic C in the paired fumigated and non-fumigated extracts using a conversion factor k_{EC} of 0.45 (Eq. 3) (Joergensen 1996).

$$\mathrm{MBC} = \left(\mathrm{TOC}_{\mathrm{Fumigated}} - \mathrm{TOC}_{\mathrm{Non-fumigated}}\right)/k_{\mathrm{EC}} \quad (3)$$

where $\mathrm{TOC}_{\mathrm{Fumigated}}$ is TOC of fumigated soil sample and $\mathrm{TOC}_{\mathrm{Non\text{-}fumigated}}$ is TOC of the paired non-fumigated soil sample. We further determined the ^{13}C-enrichment of MBC using a method outlined above for determining the ^{13}C-enrichment of DOC (Garcia-Pausas and Paterson 2011) and calculated the fractions of MBC derived from plant and SOM using Eqs. 1 and 2.

Statistical and genetic analyses

The software package GenStat (Eighteenth Edition, VSN International Ltd) was used for all statistical analyses. Repeated-measures analysis of variance (ANOVA) was used to assess the effects of barley genotype and sampling date on soil solution DOC (i.e. total DOC, root-derived DOC and SOM-derived DOC), with barley genotype as the fixed factor and sampling date as the repeated factor. In addition, one-way ANOVA was used to test for differences in soil MBC and plant biomass (including root and shoot biomass) among genotypes at harvest point. Where statistically significant ($p < 0.05$) genotype effects were found, the least significant difference (LSD) was used to assess differences between individual means.

For identification of barley chromosome regions influencing the observed phenotypes (i.e. root-derived MBC, root-derived DOC and root biomass, all at 39d), one-way ANOVA was used to analyse the genotype means for each trait to test whether these were related to the genotype for each SNP on the map, with 235 SNPs used in the analysis. Each of these markers represents a block of contiguous SNPs that were found polymorphic for the same RCSLs. We removed redundant markers from the initial group of 1848 SNPs used to characterise the RCSLs. Nevertheless, the mapping information for the group of SNPs within each block was considered to define the QTL regions as explained in de la Fuente Cantó et al. (2018). We present all marker effects that are significant with $p < 0.05$, but we focus on the most significant ones with $p < 0.01$ to take into account that multiple markers have been tested. The relative performance of individual RCSLs ($\mathrm{RP}_{\mathrm{RCSL}}$), in comparison to the lines with the genotype of the elite parent Harrington, was calculated following Eq. 4. Caesarea 26-24 (donor parent) was not used in the genetic analysis.

$$\mathrm{RP}_{\mathrm{RCSL}} = \left[\left(\mathrm{M}_{\mathrm{RCSL}} - \mathrm{M}_{\mathrm{Harrington}}\right)/\mathrm{M}_{\mathrm{Harrington}}\right] \times 100 \quad (4)$$

where $\mathrm{M}_{\mathrm{RCSL}}$ is the trait mean of the RCSL genotypes and $\mathrm{M}_{\mathrm{Harrington}}$ is the trait mean of the lines with the genotype of the elite parent.

Where two phenotypes or traits were found to be influenced by the same chromosome region (i.e. multiple marker effects), Pearson correlation was used to determine the relationship between those two phenotypes. Pearson correlation was also applied to determine the relationship between root biomass and root-derived MBC.

Identification of candidate genes

Genes which are located within the introgressed chromosome regions that span the observed significant markers and their physical positions were determined from the gene database Barlex (https://apex.ipk-gatersleben.de/apex/f?p=284:10) and a map-based barley genome assembly with high-confidence genes (Mascher et al. 2017). Based on gene functional annotation, in relation to root-derived MBC and DOC subsets of potential candidates were identified.

Results

Phenotypes

The barley plants were at vegetative (i.e. tillering) stage at the harvest date, 39d after planting, and showed no signs of stress (i.e. pest or pathogen infestation, nor nutrient or water deficiency). Plant tissue ^{13}C-enrichment ranged from 2.06 to 2.17 atom% ^{13}C, and did not significantly differ among genotypes. Statistically significant ($p < 0.05$) differences in root biomass (Fig. 1) and shoot biomass (Fig. S1) among the genotypes were observed.

For soil related characteristics, ANOVA (Tables 1 and S1) showed significant ($p < 0.05$)

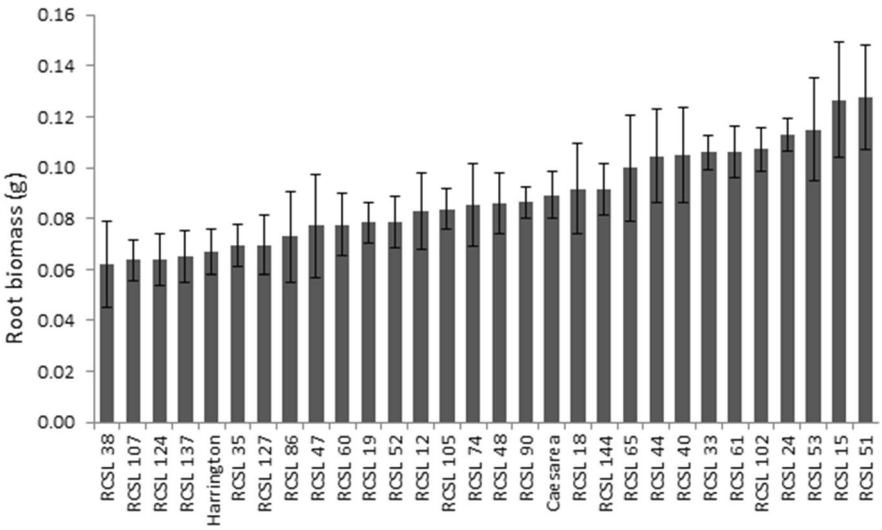

Fig. 1 Root biomass (dry weight) measured at 39d for 28 barley recombinant chromosome substitution lines (RCSLs) and parental genotypes Caesarea 26-24 and Harrington. Values are means ($n = 3$). Bars show ± one standard error of the mean. Significant differences ($p < 0.05$) among barley genotypes were estimated using ANOVA

Table 1 Analysis of variance for barley plant biomass, soil microbial biomass carbon (MBC) and dissolved organic carbon (DOC) in soil solution

Parameter	Source of variation	df	*p* value
Root biomass (g)	Barley genotype	29	**0.041**
Shoot biomass (g)	Barley genotype	29	**0.001**
Total plant biomass (g)	Barley genotype	29	**0.019**
Root-derived MBC (mg C kg^{-1} soil)	Barley genotype	29	**0.013**
SOM-derived MBC (mg C kg^{-1} soil)	Barley genotype	29	0.887
Total MBC (mg C kg^{-1} soil)	Barley genotype	29	0.854
Root-derived DOC (mg C L^{-1} soil solution)	Barley genotype	29	**0.001**
	Sampling time	1	**< 0.001**
	Genotype × time	29	**0.006**
SOM-derived DOC (mg C L^{-1} soil solution)	Barley genotype	29	0.257
	Sampling time	1	**< 0.001**
	Genotype × time	29	0.984
Total DOC (mg C L^{-1} soil solution)	Barley genotype	29	0.357
	Sampling time	1	**< 0.001**
	Genotype × time	29	0.974

Significant *p* values ($p < 0.05$) are shown in bold

DOC was measured at 30d and 39d, while plant biomass and MBC were measured at 39d

df degrees of freedom, *SOM* soil organic matter

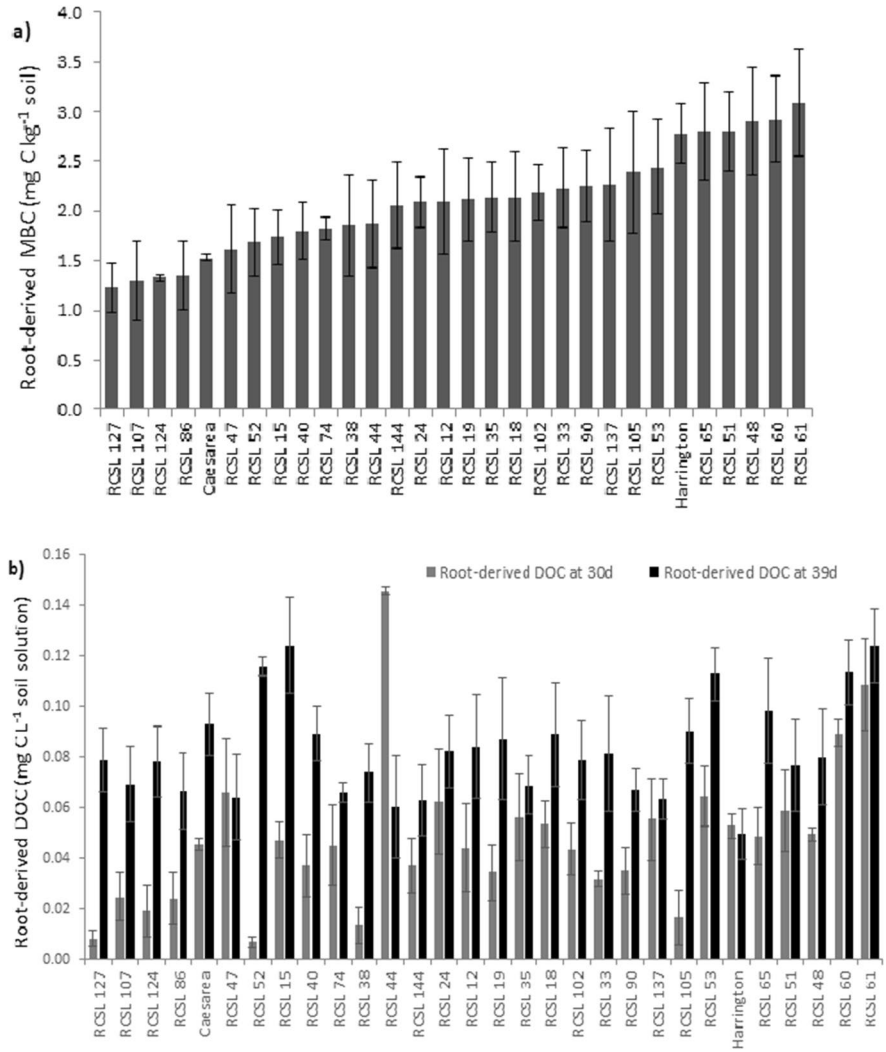

Fig. 2 Root-derived microbial biomass-C (MBC) measured at 39d (**a**) and root-derived dissolved organic-C (DOC) measured at 30d and 39d (**b**) for 28 barley recombinant chromosome substitution lines (RCSLs) and parental genotypes Caesarea 26-24 and Harrington. Values are means ($n=3$). Bars show ± one standard error of the mean. Significant differences ($p<0.05$) among barley genotypes and between sampling times were estimated using ANOVA

variation among genotypes for root-derived MBC (Fig. 2a) and root-derived DOC (Fig. 2b). The largest root-derived MBC, measured at 39d, was determined as 3.1 mg C kg^{-1} soil, while the smallest root-derived MBC was estimated as 1.2 mg C kg^{-1} soil. The estimated root-derived DOC among genotypes ranged from 0.01 to 0.15 mg C L^{-1} soil solution (0.05 mg C L^{-1} soil solution average) at 30d and 0.05–0.12 mg C L^{-1} soil solution (0.08 mg C L^{-1} soil solution average) at 39d, showing an increasing trend over time. There were no significant differences (Table 1) in total or SOM-derived MBC and total or SOM-derived DOC among the genotypes. Average SOM-derived MBC and average total MBC were 68.20 and 70.30 mg C kg^{-1} soil, respectively. SOM-derived DOC decreased from an average of 19.97 mg C L^{-1} soil solution at 30d to 17.11 mg C L^{-1} soil solution at 39d, while total DOC decreased from an average of 20.12 mg C L^{-1} soil solution at 30d to 17.20 mg C L^{-1} soil solution at 39d.

Pearson correlation showed a moderate, positive relationship between root-derived DOC and root-derived MBC ($r=0.52$; $p<0.001$) (Fig. 3a) and a low, positive correlation between root biomass and root-derived MBC ($r=0.44$; $p<0.001$) (Fig. 3b).

Genetic marker effects and annotated genes

In total, 16 statistically significant ($p<0.05$) marker effects (including two multiple marker effects) for root-derived MBC and root-derived DOC, both at

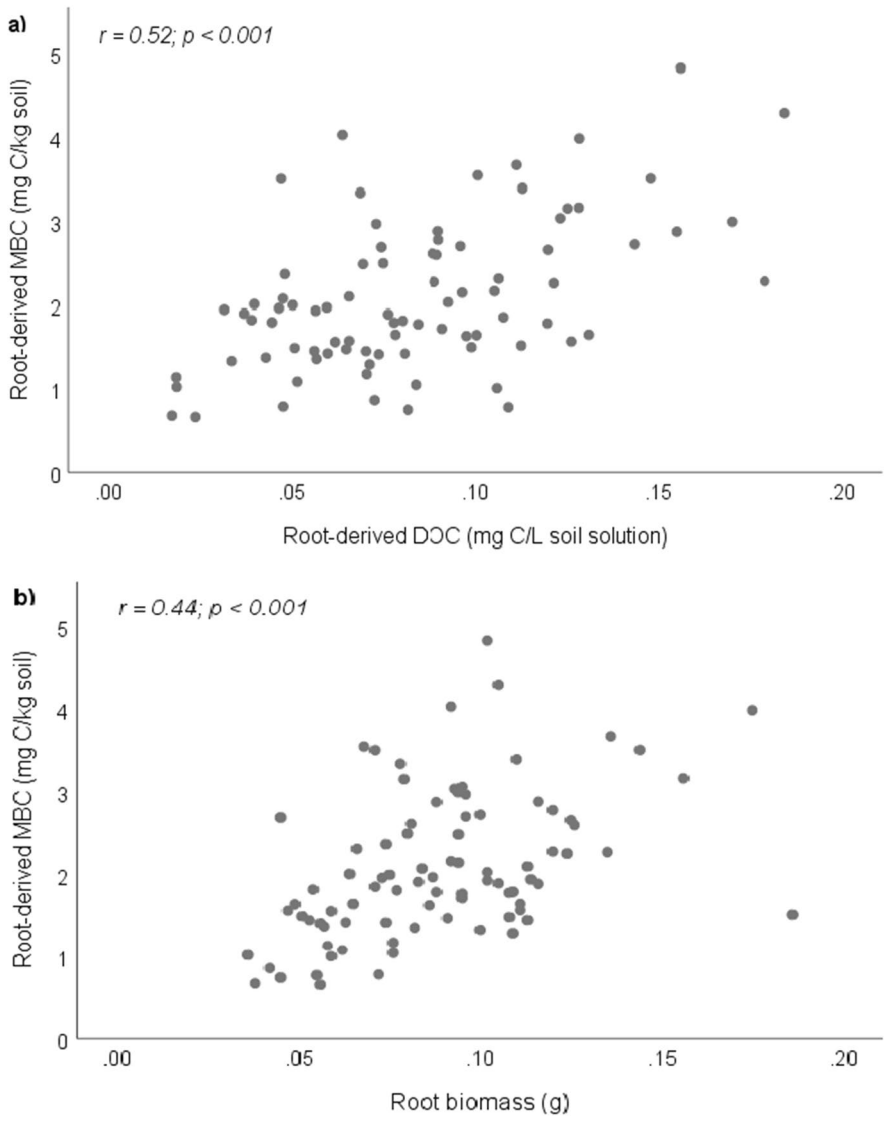

Fig. 3 Correlation between root-derived dissolved organic-C (DOC) and root-derived microbial biomass-C (MBC) (**a**) and that between root biomass and root-derived MBC (**b**), all measured at 39d, for 28 barley Recombinant Chromosome Substitution Lines (RCSLs) and parental genotypes Caesarea 26-24 and Harrington. Each point in the scatter plot represents paired (root-derived DOC and MBC) individual pot measurements

39d, were detected (Table 2). Root-derived MBC had the highest number of significant marker effects. These marker effects were found on chromosomes 2H, 3H, 4H and 7H. Five of the markers (12_31293, SCRI_RS_129857 and SCRI_RS_171032 on 2H, SCRI_RS_183659 on 3H and 11_21437 on 7H, representing block regions containing 39, 64, 33, 248 and 118 annotated and unknown genes, respectively) were highly significant ($p \leq 0.01$) (Table 2). Several genes encoding proteins involved in metabolite transport (e.g. HORVU2Hr1G091190.3 for sugar transporter 9 around 647,278,826–647,282,664 bp on 2H and HORVU3Hr1G074290.4 for MATE efflux family protein around 557,949,641–557,952,693 bp on 3H) were found around these marker positions. Furthermore, genes/proteins involved in gene expression regulation (e.g. HORVU2Hr1G090640.1 for TCP family transcription factor around 645,146,133–645,147,494 bp and HORVU2Hr1G092030.14 for NAC domain protein around 650,452,493–650,457,428 bp on 2H) and metabolite synthesis (e.g. HORVU3Hr1G075870.1 for photosystem II reaction center W protein around 566,531,044–566,534,103 bp on 3H and HORVU7Hr1G012380.5 for starch synthase 2 around 17,089,868–17,094,404 bp on 7H) were found on these chromosome regions (Table S2). On one chromosome region, at marker SCRI_RS_200957 on 4H (position 76.3–78.5 cM, representing a region

Table 2 Statistically significant ($p < 0.05$) marker block regions for root-derived microbial biomass-C (MBC) and root-derived dissolved organic-C (DOC) detected in 28 barley recombinant chromosome substitution lines (RCSLs) derived from a cross between a wild donor (Caesarea 26-24) and an elite recurrent parent (Harrington)

Trait	Marker[a]	Chr	Block Position[b] (cM)	Prob. snp	R^2	M_{RCSL}	$M_{Harrington}$	SED	RP (%)	Physical position (bp)	Interval (bp)	Number of genes
Root-derived MBC at 39d	12_31293*	2H	73.7–75.2	0.008	20.33	2.72	2.02	0.25	35.0	641,328,117	640,849,650–645,123,440	39
	SCRI_RS_129857*	2H	75.2–79.4	0.001	29.20	2.74	1.98	0.21	38.0	645,575,740	645,123,440–649,449,402	64
	SCRI_RS_171032*	2H	79.4–80.0	0.008	20.25	2.72	2.02	0.25	34.9	650,676,570	649,449,402–652,031,329	33
	SCRI_RS_110693	3H	46.2–49.3	0.015	17.21	2.95	2.05	0.34	43.6	75,453,978	47,570,630–101,184,493	377
	11_10380	3H	49.3–51.2	0.028	13.57	2.72	2.04	0.29	33.1	142,551,916	101,184,493–169,952,332	373
	SCRI_RS_165264	3H	51.3–58.6	0.028	13.49	2.72	2.04	0.29	33.0	353,175,383	212,003,876–515,466,709	1282
	11_10335‡	3H	58.6–61.8	0.015	17.12	2.94	2.05	0.34	43.5	517,015,889	515,466,709–535,469,596	181
	SCRI_RS_138291‡	3H	62.5–62.7	0.015	17.12	2.94	2.05	0.34	43.5	540,504,773	535,469,596–541,043,610	39
	SCRI_RS_183659*	3H	62.7–69.0	0.002	27.58	2.93	2.02	0.27	45.1	541,043,610	541,043,610–566,989,034	248
	11_20093	3H	69.0–73.0	0.033	12.61	2.85	2.06	0.35	38.4	567,582,668	566,989,034–574,657,494	98
	SCRI_RS_200957	4H	76.3–78.5	0.019	15.71	1.63	2.21	0.23	−26.1	590,413,614	586,059,894–590,413,750	56
	SCRI_RS_132017	7H	9.1–12.7	0.034	12.47	2.61	2.03	0.26	28.4	11,427,761	1,904,888–14,802,282	384
	11_10841	7H	12.7–13.9	0.019	15.64	2.76	2.04	0.29	35.1	14,802,282	14,802,282–16,564,325	53
	11_21437*	7H	13.9–20.4	0.003	26.23	2.79	2.00	0.24	39.2	16,564,325	16,564,325–22,774,581	118
	11_20495	7H	20.4–23.7	0.024	14.39	2.53	2.01	0.22	26.0	22,774,581	22,774,581–26,829,636	72

Table 2 (continued)

Trait	Marker[a]	Chr	Block Position[b] (cM)	Prob. snp	R^2	M_{RCSL}	$M_{Harrington}$	SED	RP (%)	Physical position (bp)	Interval (bp)	Number of genes
Root-derived DOC at 39d	11_10357	1H	95.9–100.9	0.029	13.41	0.12	0.08	0.02	52.9	517,540,377	509,765,620—528,031,794	136
	11_10335‡	3H	58.6–61.8	0.031	12.98	0.11	0.08	0.01	37.9	517,015,889	515,466,709–535,469,596	181
	SCRI_RS_138291‡	3H	62.5–62.7	0.031	12.98	0.11	0.08	0.01	37.9	540,504,773	535,469,596–541,043,610	39

Markers marked with a dagger (‡) were significant for both traits. Those marked with a star (*) showed marker-trait associations with $p \leq 0.01$. R^2 is the proportion of phenotypic variance explained by the quantitative trait loci (QTL). $M_{Harrington}$ is the trait mean of the lines with the genotype of the elite parent Harrington, M_{RCSL} is the trait mean of the RCSL lines with the alternative SNP genotype, SED is the standard error of difference, and RP is the relative performance (%) of individual RCSLs in comparison to the lines with the genotype of the elite parent. The gene numbering is according to the 2017 annotation

[a]Marker representing a block of contiguous SNP markers

[b]Marker block region established by the genetic position corresponding to the first and the last SNP markers defining the block

spanning 586,059,894–590,413,750 bp containing 56 genes), the exotic (Caesarea 26-24) introgression decreased the root-derived MBC (by 26%). For all other significant markers or chromosome regions, exotic introgressions increased the root-derived MBC by 26–45% (Tables 2 and 3). Three significant marker effects were detected for root-derived DOC, one on chromosome 1H and two on chromosome 3H. Here, the two markers identified on chromosome 3H (11_10335 on position 58.6–61.8 cM spanning 515,466,709–535,469,596 bp with 181 genes including the gene (HORVU3Hr1G068450.2) encoding trehalose-6-phosphate (T6P) phosphatase, and SCRI_RS_138291 on position 62.5–62.7 cM spanning 535,469,596–541,043,610 bp with 39 genes) were also detected for root-derived MBC (Tables 2 and S2). For these two multiple marker effects, exotic introgressions increased root-derived DOC and root-derived MBC by 38 and 44%, respectively.

Seven statistically significant ($p < 0.05$) markers were identified for root biomass (Table 4). These marker effects were found on chromosomes 2H, 3H, 5H and 7H, with the exotic introgressions increasing root biomass by 29–36%.

A barley genetic map showing statistically significant markers identified for root-derived MBC, root-derived DOC and root biomass is presented in Fig. 4.

Discussion

Phenotypic evaluation

Analysis of soil microbial biomass showed that concentration of root-derived MBC varied among genotypes. Total MBC and its proportion derived from SOM did not significantly differ among the genotypes. The lack of significant differences in total MBC among genotypes (notwithstanding that root-derived MBC varied among the genotypes) may suggest that barley rhizodeposition impacted microbial activity, including microbial use of root-derived C, rather than microbial growth. This assumption agrees with Shahzad et al. (2015) who showed that root exudation by grassland species could stimulate microbial activity without increasing microbial biomass. It also concurs with Paterson et al. (2008) who suggested that smaller C inputs to soil may not cause significant

Table 3 Ranking of barley recombinant chromosome substitution lines (RCSLs) and the control parent Harrington based on measured root-derived microbial biomass-C (MBC) versus most significant ($p < 0.05$) markers for root-derived MBC

SCRI_RS_200957	Marker[a]	12_31293	SCRI_RS_129857	SCRI_RS_171032	SCRI_RS_110693	11_10380	SCRI_RS_165264	11_10335	SCRI_RS_138291	SCRI_RS_183659	11_20093	SCRI_RS_132017	11_10841	11_21437	11_20495	Root-derived MBC (mg C kg^{-1} soil)*
4H	Chr	2H	2H	2H	3H	3H	3H	3H	3H	3H	3H	7H	7H	7H	7H	
76.3	Position[b] (cM)	73.7	75.2	79.4	46.2	49.3	51.3	58.6	62.5	62.7	69	9.1	12.7	13.9	20.4	
− 26%	RP	35%	38%	35%	44%	33%	33%	44%	44%	45%	38%	28%	35%	39%	26%	
1	RCSL 127	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.23
1	RCSL 107	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.30
0	RCSL 124	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.33
0	RCSL 86	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.35
0	RCSL 47	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.61
1	RCSL 52	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.68
1	RCSL 15	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.73
1	RCSL 40	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.80
0	RCSL 74	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.82
1	RCSL 38	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.85
1	RCSL 44	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	1.87
0	RCSL 144	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.06
1	RCSL 24	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.09
1	RCSL 12	**0**	**0**	**0**	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.09
1	RCSL 19	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.12
1	RCSL 35	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.14
1	RCSL 18	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.14
1	RCSL 102	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	**0**	1	1	**0**	2.18
1	RCSL 33	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	**0**	2.24
1	RCSL 90	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	**0**	**0**	**0**	1	2.25
1	RCSL 137	*1*	*1*	*1*	*1*	**0**	**0**	1	1	1	1	1	1	1	1	2.27
1	RCSL 105	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.39
1	RCSL 53	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.44
1	Harrington	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.77

Table 3 (continued)

SCRI_RS_200957	Marker[a]	12_31293	SCRI_RS_129857	SCRI_RS_171032	SCRI_RS_110693	11_10380	SCRI_RS_165264	11_10335	SCRI_RS_138291	SCRI_RS_183659	11_20093	SCRI_RS_132017	11_10841	11_21437	11_20495	Root-derived MBC (mg C kg^{-1} soil)*
4H	Chr	2H	2H	2H	3H	3H	3H	3H	3H	3H	3H	7H	7H	7H	7H	
76.3	Position[b] (cM)	73.7	75.2	79.4	46.2	49.3	51.3	58.6	62.5	62.7	69	9.1	12.7	13.9	20.4	
−26%	RP	35%	38%	35%	44%	33%	33%	44%	44%	45%	38%	28%	35%	39%	26%	
1	RCSL 65	*1*	**0**	**0**	*1*	*1*	**0**	**0**	**0**	**0**	**0**	*1*	*1*	*1*	*1*	2.80
1	RCSL 51	**0**	**0**	1	**0**	**0**	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	2.80
1	RCSL 48	*0*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	**0**	**0**	*1*	*1*	*0*	*0*	2.90
1	RCSL 60	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	**0**	**0**	**0**	**0**	2.93
1	RCSL 61	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	*1*	**0**	**0**	**0**	**0**	3.09

Relative performance (RP, %) of individual RCSLs, in comparison to the control parent, and the presence of the control parent genome (1, Italic) or introgressed genome region (0, Bold) from the donor parent Caesarea 26-24 are indicated

[a]Marker representing a block of contiguous SNP markers

[b]Chromosome position corresponding to the first SNP marker defining a block of contiguous markers

*SED = 0.51

changes in the microbial population, and would be processed by those organisms already present in soil. Moreover, this result is consistent with our previous study (Mwafulirwa et al. 2016) where a small number of genotypes from the same barley population did not vary in total MBC and SOM-derived MBC but varied in root-derived MBC, root-derived CO_2–C, SOM-derived CO_2–C and total CO_2–C surface soil fluxes.

The present results also did not show genotype effects on SOM-derived DOC in soil solutions sampled at 30d and 39d. However, the fraction of total DOC derived from roots varied among the genotypes at both sampling dates. Likewise, these results corroborate our earlier work (Mwafulirwa et al. 2016) where root-derived DOC varied among genotypes (that were selected from the barley population used in the present study) while SOM-derived DOC also did not vary among the genotypes. Root-derived DOC increased over time, consistent with plant growth increasing root inputs to soil. In contrast, SOM-derived DOC and total DOC decreased over time, in line with depletion of the available SOM stock. In the present study, it was also noted that concentrations of root-derived DOC among genotypes at 30d did not correlate with concentrations at 39d. This could be explained by restricted root growth because of the use of small pots, in which root growth rates may change with changes in soil nutrients (Marschner 1995) or when all the readily available soil has been explored (Garnett et al. 2009). As such, it is likely that the root-to-soil ratio for individual genotypes varied between the two sampling dates (i.e. 30d and 39d). This potentially affected exudation amounts and thus root-derived DOC concentrations in the soil solutions between those time points. Nevertheless, the pattern of root-derived DOC among genotypes at 30d was consistent with that observed in our previous work using a small number of RCSLs (Mwafulirwa et al. 2016).

Wild barley Caesarea 26-24 had a larger root biomass relative to modern barley Harrington. Indeed, detection of marker effects in the RCSLs (discussed below) showed that the wild barley genome increased root biomass (Table 4). These findings are in agreement with the general understanding that wild barley accessions have an inherent ability to develop vigorous or extensive rooting systems (White et al. 2009; Naz et al. 2014),

Table 4 List of seven statistically significant ($p < 0.05$) marker block regions for root biomass detected in 28 barley recombinant chromosome substitution lines (RCSLs) derived from a cross between a wild donor (Caesarea 26-24) and an elite recurrent parent (Harrington)

Marker[a]	Chr	Block position[b] (cM)	Prob. snp	R^2	M_{RCSL}	$M_{Harrington}$	SED	RP (%)
11_20173	2H	38.1–40.8	0.018	15.95	0.11	0.09	0.01	31.6
SCRI_RS_14801	2H	48.4–53.8	0.014	17.39	0.11	0.09	0.01	29.1
SCRI_RS_119379	3H	3.1–8.9	0.016	16.57	0.11	0.09	0.01	28.5
SCRI_RS_110693	3H	46.2–49.3	0.028	13.63	0.12	0.09	0.01	35.3
SCRI_RS_205235	5H	55.7–71.7	0.017	16.31	0.11	0.09	0.01	31.9
SCRI_RS_120015*	7H	134.2–140.4	0.007	22.02	0.12	0.09	0.01	35.1
SCRI_RS_158599*	7H	140.4–140.9	0.006	22.14	0.12	0.09	0.01	36.4

Those marked with a star (*) showed marker-trait associations with $p \leq 0.01$. R^2 is the proportion of phenotypic variance explained by the quantitative trait loci (QTL). $M_{Harrington}$ is the trait mean of the lines with the genotype of the elite parent Harrington, M_{RCSL} is the trait mean of the RCSL lines with the alternative SNP genotype, SED is the standard error of difference, and RP is the relative performance (%) of individual RCSLs in comparison to the lines with the genotype of the elite parent

[a]Marker representing a block of contiguous SNP markers

[b]Marker block region established by the genetic position corresponding to the first and the last SNP markers defining the block

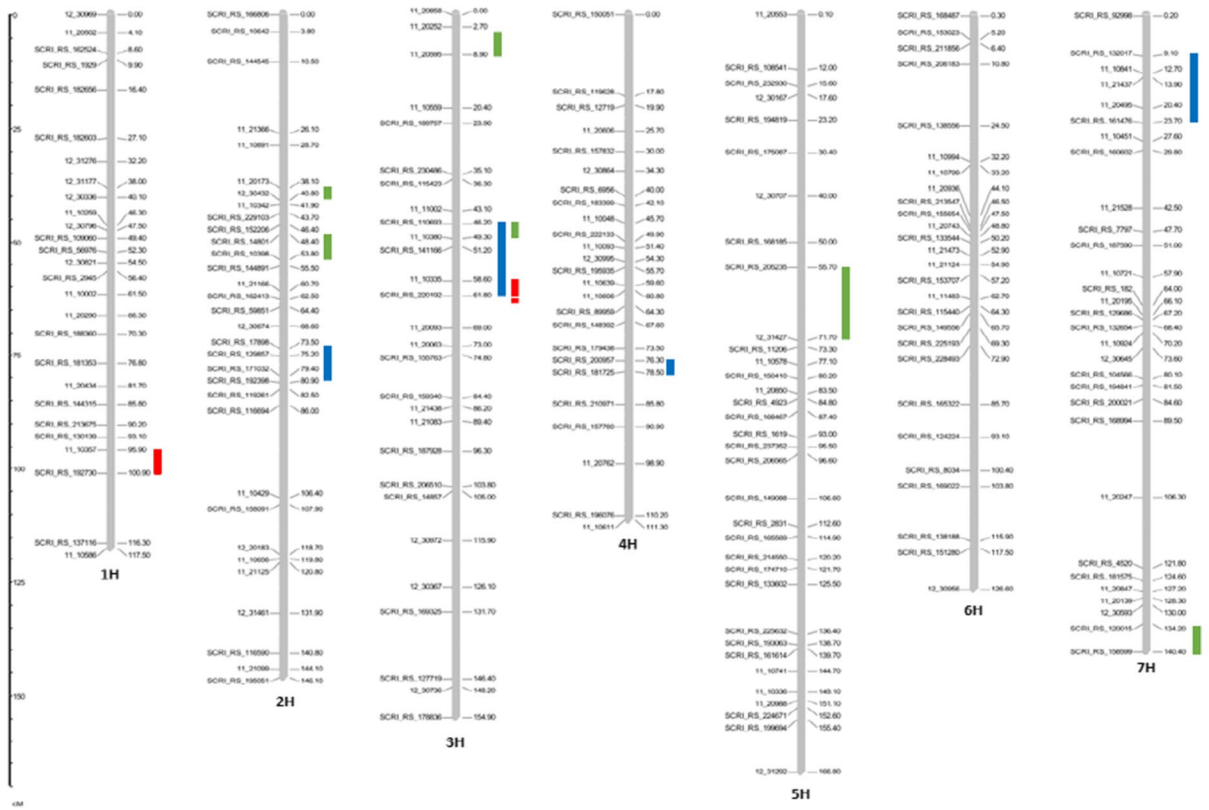

Fig. 4 Barley genetic map showing chromosome regions found associated with root-derived MBC at 39d (blue bars), root-derived DOC at 39d (red bars) and root biomass (green bars). Marker names and genetic positions (cM) correspond to the iSelect SNP chip (Comadran et al. 2012) colour figure online

that lead to greater contact between roots and soil, which in turn enhance water uptake, and thus tolerance to drought (Gahoonia and Nielsen 2004). Naz et al. (2014) also found a vigorous root system in wild barley ISR42-8 in comparison to modern barley Scarlett under control and drought conditions.

Chromosome regions and potential candidate genes influencing soil related traits

Identification of marker effects revealed 16 chromosome regions influencing root-derived MBC and root-derived DOC in soil. Of these, five chromosome regions on marker positions 73.7–75.2 cM, 75.2–79.4 cM (peak marker-trait association) and 79.4–80.0 cM on chromosome 2H (considered as single QTL), 62.7–69.0 cM on chromosome 3H and 13.9–20.4 cM on chromosome 7H showed stronger effects. The genes associated with proteins involved in metabolite synthesis (e.g. the starch synthase 2, Patterson et al. 2018) and transport (e.g. the sugar transporter 9 and the MATE efflux family protein, dos Santos et al. 2017; Julius et al. 2017) and gene expression regulation specific for roots (e.g. the TCP family transcription factor and the NAC domain protein, Janiak et al. 2019) may be potential candidate genes that influenced the variations found for root-derived MBC and DOC.

For one marker localised on chromosome 4H, position 76.3–78.5 cM, the wild barley genome introgression decreased root-derived MBC. This is in line with the phenotype data considering that the wild barley (Caesarea 26-24) (Fig. 2a) and the RCSLs with the wild barley genome on this chromosome region (Table 3) were associated with smaller root-derived MBC, relative to modern barley (Harrington). Therefore, this result suggests that Caesarea 26-24 harbours alleles on chromosome 4H that influence microbial use of root-derived C and its flow through the microbial biomass. All other detected marker effects for root-derived MBC showed that the wild barley genome introgressions increased root-derived MBC, but this was in contrast to the observed phenotype (discussed above). This, however, may be indicative of interactive allele or loci effects (Li et al. 2010), or that those effects on MBC were conferred by the modern barley (Harrington) genome. For DOC, the detected marker effects showed that the wild barley genome introgressions increased root-derived DOC. Here, all the detected marker effects (on positions 95.9–100.9 cM on chromosome 1H and 58.6–61.8 cM and 62.5–62.7 cM on chromosome 3H) were in agreement with the phenotype data. This is because the wild genotype Caesarea 26-24 was associated with larger root-derived DOC concentration (at 39d) in comparison to the modern genotype Harrington. As such, these results suggest that the wild barley genotype Caesarea 26-24 carries alleles that influence root-derived DOC on those three chromosome regions. This assumption is in line with the gene encoding T6P found on chromosome 3H, which could be a candidate gene influencing root C deposition and, in turn, root-derived DOC and MBC concentrations in soil. The T6P signalling system is a major regulator of resource allocation (e.g. C allocation and utilization in plants) and has been implicated in several processes in crop plants including assimilate partitioning and source-sink relationships (Paul et al. 2018, 2020). To our knowledge, this study is the first to demonstrate the localization of the barley genetic influence on soil MBC and DOC, and thus on soil C cycling.

The two genome regions on chromosome 3H (discussed above, although this could be considered a single QTL) affected both root-derived DOC and root-derived MBC. This is consistent with the relationship between these two traits, as determined by Pearson correlation analysis, being significant. Pearson correlation also showed a significant but low, positive relationship between root biomass and root-derived MBC. It is known that soil microbes acquire C (including root-derived C) in the form of DOC in the soil solution (Smolander and Kitunen 2002; Montaño et al. 2007), and that quantity of root exudation in barley plants is closely correlated with root biomass production (Darwent et al. 2003). Thus, our results support the likely links between root-derived MBC and root biomass or root-derived DOC.

However, moderate and low correlations between root-derived DOC and MBC and root biomass and root-derived MBC, respectively, suggest that root-derived MBC size was not mainly affected by root biomass size or DOC concentration. In previous work (Mwafulirwa et al. 2016), we showed that the activity (i.e. functional diversity) of soil microbes was affected by barley variety, likely due to differences in rhizodeposit quality (i.e. chemical composition) among the varieties or genotypes, which included genotypes

used in the present study. This may lead us to assume that the variation in root-derived MBC observed here among the barley genotypes was also likely due to differences in rhizodeposit chemical composition among the genotypes, and that the marker effects for root-derived MBC or DOC revealed in this work were also associated with rhizodeposit composition. There are reports for QTLs and candidate genes influencing rhizodeposit chemical composition in other crop plants (Yan et al. 2004; Hongni et al. 2011; Qiu et al. 2014; Ramongolalaina et al. 2018). For instance, Hongni et al. (2011) mapped QTLs for three root exudates related to phosphorus efficiency in maize, while Yan et al. (2004) identified QTLs influencing root exudation of organic acids in common bean.

Chromosome regions affecting root biomass

Seven significant marker effects, on chromosomes 2H, 3H, 5H and 7H, were identified for root biomass. Others have also reported the localization of QTLs influencing root biomass in barley on these chromosomes (reviewed by Sallam et al. 2019). For example, Naz et al. (2014) used the 1536-SNP barley BOPA1 set and detected a total of 13 QTLs for root dry weight and root volume using wild barley introgression lines (developed by crossing a German spring cultivar Scarlett and a wild accession ISR42-8), of which seven QTLs were located on chromosomes 2H, 3H, 5H and 7H. Another study using barley lines genotyped using the Illumina 1536-SNP array (Arifuzzaman et al. 2014) also identified seven QTLs for root dry weight, with four of the QTLs localized on chromosomes 2H, 3H, 5H and 7H. In particular, Arifuzzaman et al. (2014) detected a QTL on 2H spanning from 38.9 to 66.0 cM, corroborating our present study showing significant marker effects on the same chromosome region (38.1–53.8 cM in the present study). This correspondence with previous studies adds additional confidence in our identification of barley chromosome regions influencing MBC and DOC pools in soil.

Conclusions

Our study is the first to identify barley chromosome regions and potential candidate genes influencing MBC and DOC concentrations in soil, and thus microbial populations and C cycling in soil. In total, 16 chromosome regions were identified for root-derived MBC and DOC, mainly on chromosomes 2H, 3H and 7H. Two locations on 3H influenced both traits (i.e. root-derived MBC and DOC). In addition, one chromosome region on 4H with the wild barley genome decreased root-derived MBC, and three on chromosomes 1H and 3H increased root-derived DOC, corroborating the observed phenotypes. This work also supports the previously detected QTLs for root biomass in barley, and therefore validating the chromosome regions influencing root-derived MBC and DOC. Thus, these findings are an important step towards better understanding of the plant genetic influences on soil microbial communities, which could be vital to control C and N cycling in soil through plant breeding approaches. Further work, for example using more replications, is needed to verify the barley chromosome regions or marker effects for MBC and DOC and potential candidate genes revealed in this study, and to sequence the soil microbes to examine the interaction between plant genotype and the diversity or structure of rhizosphere microbiomes.

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Author contributions All authors contributed to the planning of this research, while LM designed and performed the experiment. LM and CAH analysed data. LM, EMB, JR, CAH and EP wrote the manuscript.

Data availability The datasets generated during the current study are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare no competing interests.

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