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# Evidence of a plant genetic basis for maize roots impacting soil organic matter mineralization

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## Abstract

Maize root traits associated with soil organic matter (SOM) mineralization were demonstrated to have a heritable genetic basis. We show root length, root diameter and cumulative root-derived C mineralization to be strong predictors of SOM-C mineralization and identify two candidate genes associated with enhanced SOM-C mineralization rates. There is potential to target these genes to enhance release of nutrients from SOM to support crop nutrition within sustainable maize cropping systems.

Keywords: maize breeding; soil organic matter mineralization; root traits; genome-wide association study (GWAS)

The maize yield gap in sub-Saharan Africa (SSA) is approximately 60-80% (van Ittersum et al. 2016). To achieve self-sufficiency by 2050, without expanding current maize area, the yield gap must be reduced to 20% (van Ittersum et al. 2016). Drought stress regularly affects maize production in SSA (Cairns and Prasanna, 2018). Low fertilizer use ( $<17 \text{ kg ha}^{-1}$ ) is another major factor contributing to this yield gap (Thierfelder et al. 2018), particularly in female-managed plots (Farnworth et al. 2017) and is exacerbated by low and variable economic returns on investment in the use of fertilizers (Jayne et al. 2018). Release of nutrients from positive priming of SOM (Kuzyakov et al. 2000) can supplement crop nutrition and increase the return on investment in fertilizer use. Breeding for maize varieties that are

able to source and utilize nutrients from SOM for their nutrition will ensure more sustainable production where practices are applied that replenish SOM, such as from stover addition.

The importance of root trait diversity in shaping plant-associated microbiome composition and nutrient cycling, including SOM mineralization, is increasingly recognized (e.g. Mwafurirwa et al. 2016; Han et al. 2020; Zai et al. 2021). Genotypic information can be used to identify genomic regions associated with these traits and narrow the selection of candidates prior to phenotype-screening (Cooper et al. 2014). Identification of potential candidate genes for traits influencing rhizosphere processes paves the way for breeding to achieve more resilient and sustainable maize production. Here we investigate the genetic basis of root traits associated with differential SOM mineralization and identify potential candidate genes.

A set of 97 elite maize lines from a drought-tolerant maize for Africa (DTMA) panel and 8 commercial hybrids were selected for screening. Genotypes were grown (29 days) under controlled environment conditions in a granitic sandy soil from Domboshawa, Zimbabwe (-17.6091 Lat, 31.13374 Long). All the genotypes were replicated two times. In addition, some of the genotypes were randomly selected and replicated a few more times (Table S5). The experimental design was a sequential randomized block design, and included unplanted controls. Exposure to  $^{13}\text{C}$ -enriched  $\text{CO}_2$  (20 atom%  $^{13}\text{C}$ ) throughout the growth period enabled quantification of root- and SOM-derived  $\text{CO}_2$ -efflux rates at 16, 23 and 29 days after planting and cumulative C mineralization over the three weeks period of  $\text{CO}_2$  measurement, following the approach of Mwafurirwa et al. (2016). The nine traits measured in this study were cumulative SOM-C mineralized (SOMC,  $\mu\text{g C g}^{-1}$  soil), cumulative root-derived C mineralized (CRM,  $\mu\text{g C g}^{-1}$  soil), average root diameter (ARD, mm), root length (RL, mm), root biomass (RBM, g), shoot biomass (SBM, g), total plant biomass (Stover, g), specific root length (SRL) and root-to-shoot biomass ratio (BM-RS). SBM and RBM were determined based on harvest dry weight (V3 growth stage) at 29 days after planting (shoots were cut at the soil surface and roots were carefully removed from the soil and washed in deionized water). Fresh roots were scanned, and root length and average root diameter measured using WINrhizo (Mwafurirwa et al. 2021). To explore the plant traits that could significantly explain the variation in SOM-C mineralization, correlation and stepwise multiple linear regression (SMLR) analyses were conducted for all genotypes (lines and hybrids) and for lines or hybrids separately. Further details of plant growth conditions,  $^{13}\text{C}$ - $\text{CO}_2$  labelling, measurements and phenotypic data are provided in Mwafurirwa et al. (2021) and supplementary materials. Details of genotyping of materials and methodology of genome-wide association study (GWAS) and genomic selection (GS) are provided in the supplementary materials.

We observed significant clustering of genotypes into six groups based on their phenotypic performance (Fig. 1; Table S1), with the maize lines clustering distinctly from the hybrid varieties (Fig. 1). For inbred lines, root diameter and root-derived soil CO<sub>2</sub> efflux (from root respiration and microbial mineralization of rhizodeposits) were positively correlated ( $r = 0.39$  and  $0.24$  respectively;  $P < 0.05$ ) with SOM mineralization rates (Fig. S1). SMLR indicated that root length, diameter, and root-derived CO<sub>2</sub> efflux together explained 47% of variation in SOM mineralization ( $P < 0.001$ ; Table S2) across inbred lines. However, for hybrids, none of these traits were significantly correlated with SOM mineralization (Fig. S1). This variation within tropical germplasm for maize root traits that correlate with SOM mineralization rates suggests that exploration of the genetic basis of inbred lines can serve as a viable option for targeted maize breeding for sustainable intensification.

Variation in SOM mineralization rates under plants is usually attributed to rhizosphere priming effects, where the exudation of organic compounds and root turnover affects microbial activity and use of SOM as substrate (Kuzyakov et al. 2000). While factors such as root physical impacts on soil structure, nutrient and water availabilities may also affect microbial activity, our results are consistent with differential priming impacts among inbred lines (Mwafulirwa et al. 2021). Increasing root diameter is commonly associated with increased rates of exudation, resulting from greater photo assimilate supply to thicker roots (Han et al. 2020; Zai et al. 2021). In turn, the magnitude of rhizosphere priming effects has been found to be proportional to the substrate supply to rhizosphere microbial communities (Paterson and Sim, 2013), suggesting the magnitude of rhizodeposition may be a key discriminating factor between inbred lines. This interpretation is also supported by the strong improvement to the variance explained when root-derived CO<sub>2</sub> efflux (partly from microbial use of rhizodeposits) is included in the SMLR with SOM mineralization (Table S2).

Genotypic variances were significant for all traits that correlated with SOM mineralization, and broad-sense heritability ranged from 0.18 to 0.65 (Table S3). Importantly, all traits showed normal distribution of best linear unbiased predictions (BLUPs) which supports their quantitative inheritance (Fig. 2A). DNA markers (199,755 markers) used for GWAS well represented the maize genome (Fig. S2A). The principal components (Fig. S2B) and kinship heatmap (Fig. S3) indicated low levels of relatedness among most pairs of inbred lines. GWAS for nine traits revealed 84 significant SNPs ( $P < 5 \times 10^{-5}$ ; Fig. 2B; Table S4). Association analyses revealed 6, 25, 3, 7, 24, 13, 8, 3 and 7 SNPs significantly associated with SOMC, CRM, SBM, RBM, RL, ARD, SRL, BM-RS and stover, respectively (Fig. 2B; Table S4). The distribution of significant SNPs across chromosomes varied between 4 in chromosome 8, to 15 in chromosome 5 (Table S3). The allelic effect (difference in mean performance for trait value between lines with the major or minor allele) for significant SNPs ranged from  $-1.27$  to  $1.43$  for SOM mineralization.

108

109 For all nine traits, a total of 23 potential candidate genes were identified (Table S4). Root-derived C  
110 mineralization was negatively correlated with SRL and associated with candidate gene  
111 *GRMZM2G141456* with functions for proteolysis, hydrolase activity and metallo-peptidase activity.  
112 Genomic prediction correlations were -0.19 and -0.08 for SOMC and CRM, respectively (Fig. 2C).  
113 Similar prediction correlations have been reported for less related populations or complex traits in maize  
114 (Riedelsheimer et al. 2013), although not for SOM mineralization. Moderate prediction correlations for  
115 other traits suggests genomic prediction can be used to improve these traits within a breeding program.

116

117 The genetic basis of variation in root exudation (amount and quality) is not well understood. In this  
118 context, we have identified two candidate genes associated with enhanced SOM mineralization rates:  
119 *GRMZM2G114362* with functions for proteolysis and peptidase activity, and *GRMZM2G170962* for  
120 proton-transporting ATP synthase and ATP synthesis, which are associated with the regulation and  
121 active release of root exudates into the rhizosphere. This suggests the potential for targeting these  
122 genes to promote root exudate release and to stimulate the microbial community to release nutrients  
123 from SOM for crop nutrition. This therefore holds great promise for enhancing sustainable production in  
124 systems managed to enhance SOM, for example through return of stover to soil which is common in  
125 conservation agriculture (Thierfelder et al. 2015), thereby promoting a more closed nutrient cycle, that  
126 increases the return on investment in inputs. Root traits associated with SOM mineralization cannot  
127 logistically be incorporated into maize breeding in SSA as this would require accurate phenotyping of  
128 thousands of lines each year. However, our results suggest the potential of molecular breeding  
129 approaches to enrich populations for markers linked with key traits associated with SOM mineralization  
130 prior to field phenotyping.

131

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138 and Wheat for Improved Livelihoods, B&MGF Investment ID INV-003439).

139

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198



199 **Figure captions**

200

201 **Figure 1.** Cluster plot showing six major groups (Ward linkage, squared Euclidean distance) of maize  
202 genotypes based on the measured traits: Cumulative SOM-C mineralized (SOMC), cumulative root-  
203 derived C mineralized (CRM), average root diameter (ARD), root length (RL), root biomass (RBM), shoot  
204 biomass (SBM), specific root length (SRL) and root-to-shoot biomass ratio (BM-RS) for 105 maize  
205 genotypes grown in granitic sandy soil (pH<sub>H2O</sub> 4.79; CEC 1.01 meq 100g<sup>-1</sup>; total C 2.98 mg g<sup>-1</sup>; total N  
206 0.24 mg g<sup>-1</sup>; sand 83.7%, silt + clay 16.3%) in controlled environment microcosms (22.5 x 5.5cm diam;  
207 65% water holding capacity; soil bulk density 1.44g cm<sup>-3</sup>) (Mwafulirwa et al. 2021). C mineralization was  
208 determined by continuous labelling with <sup>13</sup>C-enriched CO<sub>2</sub> (20 atom% <sup>13</sup>C) through a growth chamber  
209 (<10 µl L<sup>-1</sup> CO<sub>2</sub> outlet airflow; 12-hour daily photoperiod, 512µmol m<sup>-2</sup> s<sup>-1</sup> PAR). Soil CO<sub>2</sub> fluxes at 16,  
210 23 and 29 days after planting were partitioned to SOM- and root-derived C based on their δ<sup>13</sup>C  
211 signatures, following Mwafulirwa et al. (2016). Clustering formed six main groups, with lines mostly  
212 distributed in first five groups, whereas six hybrids (SC633, PGS61, Pristine 601, 30G19, PAN53 and  
213 ZAP55 in blue) are grouped together as group VI. Traits contributing high mean performance for clusters  
214 were specified on the figure. Pedigrees associated with the genotype codes can be found in Wen et al.  
215 (2011).

216

217 **Figure 2.** Phenotypic distribution of best linear unbiased predictions (BLUPs) for each trait (A), GWAS  
218 based Manhattan plots (B) and genomic prediction correlations (C) for nine traits from DTMA association  
219 panel. In the Manhattan plots each color represents different chromosome and the horizontal lines show  
220 the threshold p value at  $P < 5 \times 10^{-5}$ . SOM-C Cumulative soil organic matter C mineralized (µg C g soil<sup>-1</sup>),  
221 CRM Cumulative root-derived C mineralized (µg C g soil<sup>-1</sup>), SBM shoot biomass (g), RBM root  
222 biomass (g), RL root length (m), ARD average root diameter (mm), SRL specific root length, BM\_RS  
223 root-to-shoot biomass ratio and Stover (total plant biomass, g).

224

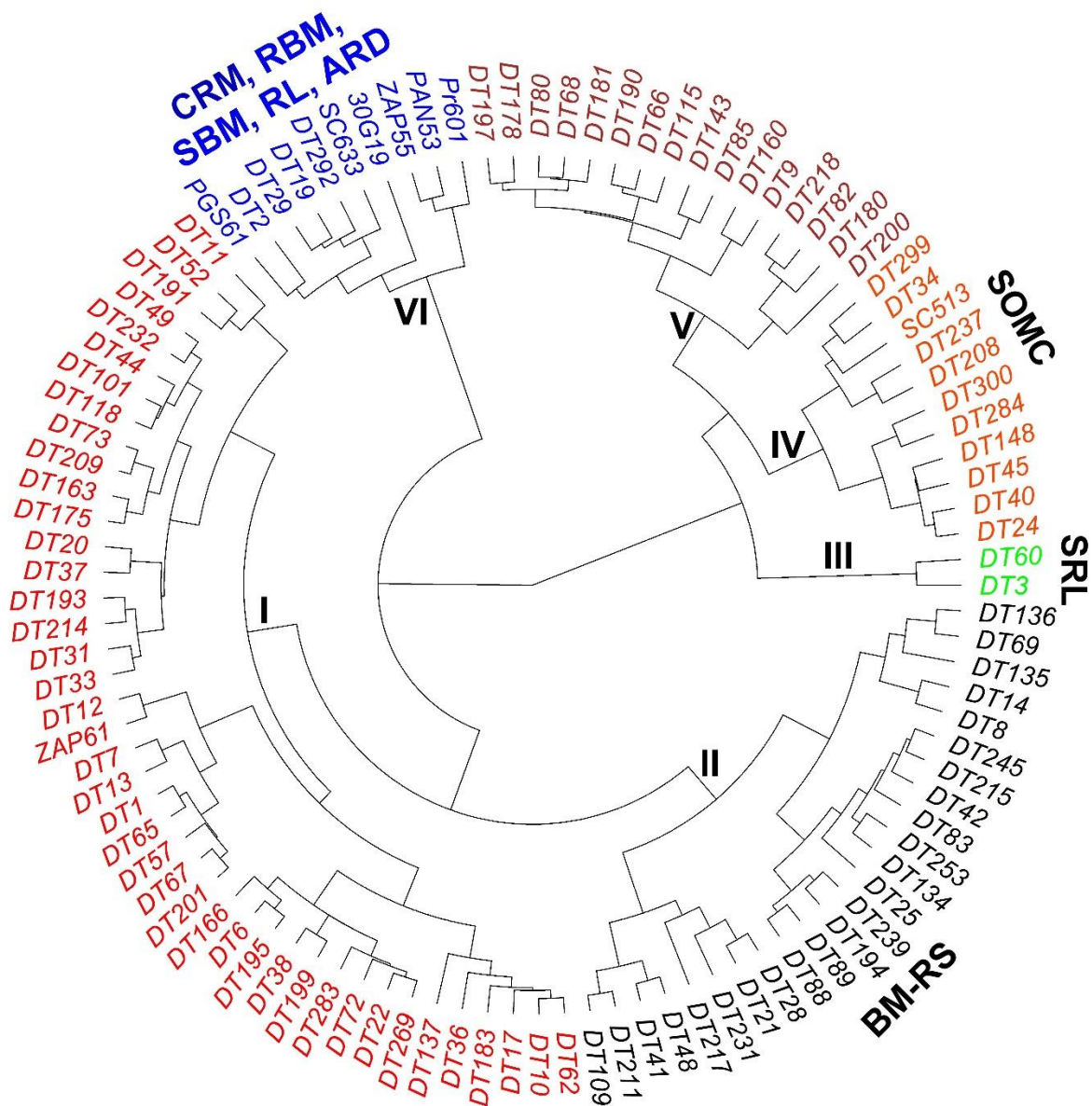
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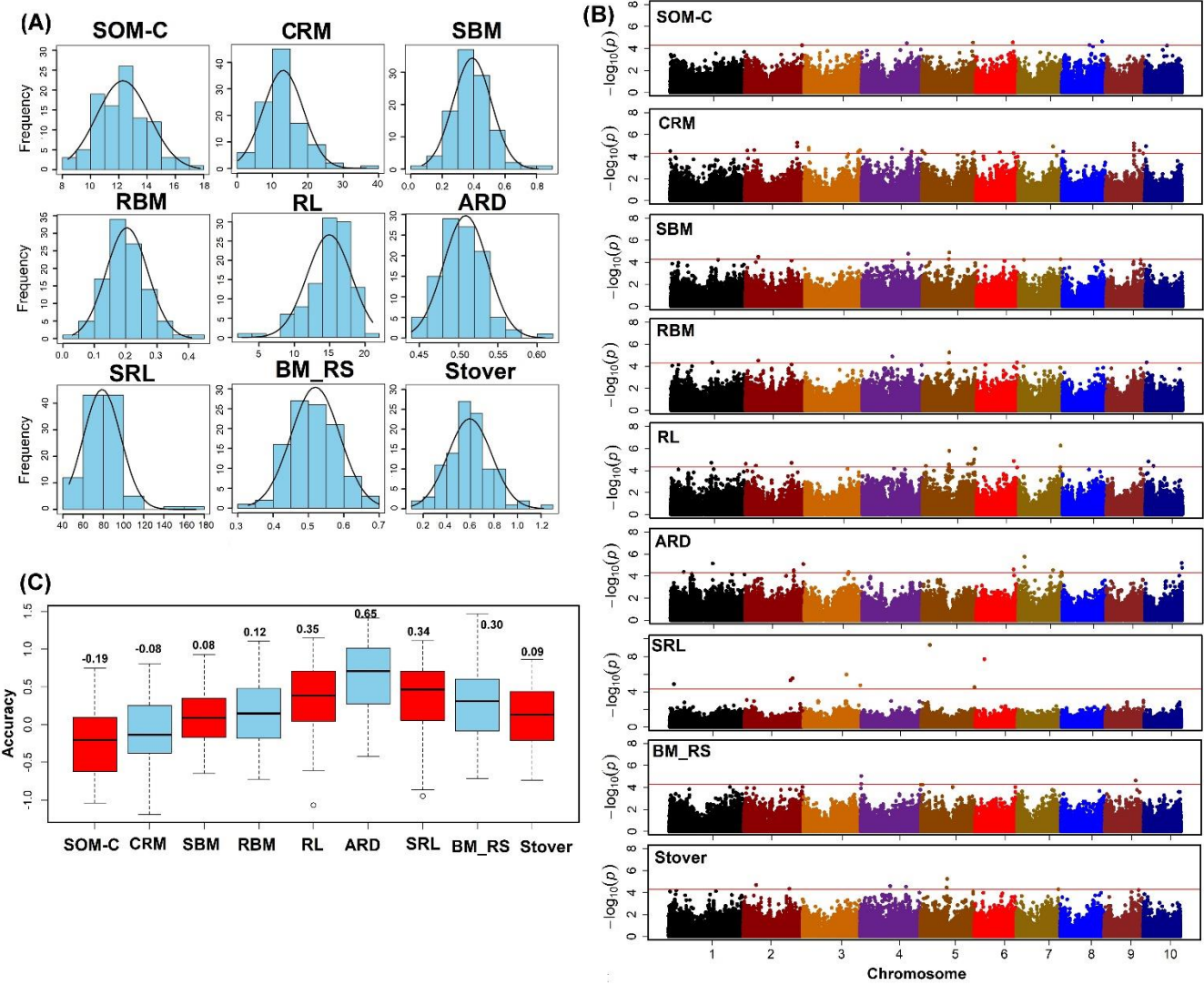


Figure 2.