



The genetic basis of the root economics spectrum in a perennial grass

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Construction economics of plant roots exhibit predictable relationships with root growth, death, and nutrient uptake strategies. Plant taxa with inexpensively constructed roots tend to more precisely explore nutrient hotspots than do those with costly constructed roots but at the price of more frequent tissue turnover. This trade-off underlies an acquisitive to conservative continuum in resource investment, described as the “root economics spectrum (RES).” Yet the adaptive role and genetic basis of RES remain largely unclear. Different ecotypes of switchgrass (*Panicum virgatum*) display root features exemplifying the RES, with costly constructed roots in southern lowland and inexpensively constructed roots in northern upland ecotypes. We used an outbred genetic mapping population derived from lowland and upland switchgrass ecotypes to examine the genetic architecture of the RES. We found that absorptive roots (distal first and second orders) were often “deciduous” in winter. The percentage of overwintering absorptive roots was decreased by northern upland alleles compared with southern lowland alleles, suggesting a locally-adapted conservative strategy in warmer and acquisitive strategy in colder regions. Relative turnover of absorptive roots was genetically negatively correlated with their biomass investment per unit root length, suggesting that the key trade-off in framing RES is genetically facilitated. We also detected strong genetic correlations among root morphology, root productivity, and shoot size. Overall, our results reveal the genetic architecture of multiple traits that likely impacts the evolution of RES and plant aboveground–belowground organization. In practice, we provide genetic evidence that increasing switchgrass yield for bioenergy does not directly conflict with enhancing its root-derived carbon sequestration.

QTL | root orders | specific root length | switchgrass | trade-off

Root uptake of belowground resources, such as water and nutrients, are fundamental to plant productivity and ecosystem carbon sequestration (1). Plants have evolved diverse adaptive strategies of root growth and resource acquisition across various environments (2–4). It is becoming clear that these root-related biological and ecological processes can be driven by simple root morphological traits. For instance, species with thinner and lower-cost roots (less biomass investment per unit root length) can more rapidly leverage resources for soil nutrient exploration, whereas species with thicker and more expensive roots are more conservative in resource acquisition but with higher-tissue persistence (5, 6). The tradeoffs between root nutrient exploration and resource conservation provide the framework of the widely hypothesized root economics spectrum (RES) among regional and world-wide plant taxa, which describes the constrained variation space of root trait relations (7, 8). The RES also likely exists among genotypes within a species (9, 10), and the position of a plant species or plant genotype occupied in the RES indicates its resource acquisition strategy, as well as its influence on ecosystem processes, such as carbon input to soil via root turnover (11, 12). Thus, understanding the relations between root form and functions, such as root growth, death, and nutrient uptake, is important

for plant health, global food security, and climate change mitigation (1, 13, 14). However, empirical evidence of RES has often been controversial, and a universal framework of RES remains largely elusive (7, 15).

Natural selection has been suggested to be one of the main driving factors shaping plant functional trait covariances (16). For instance, the trade-off between root persistence and biomass cost per unit root length has been explained by the resource optimization theory (17). This theory demonstrates that costly constructed roots tend to be long lived to ensure a favorable nutrient and water return on the high-resource investment. But long root lifespan is not always favorable because maintenance cost increases and uptake capacity decreases with root age (18). Therefore, natural selection appears to favor plants with inexpensive, ephemeral roots; expensive, long-lived roots, and roots with intermediate cost and longevity. However, plant traits are often determined by interacting genetic and environmental factors, and thus, the evolution of trait correlations can be limited not only by selection against combinations with low fitness but also by genetic constraints on trait combinations, such as pleiotropy and linkage disequilibrium (19, 20). Trait correlation caused by pleiotropy can result in biophysical linkage through the effects of shared key genes (21). For instance, genes controlling the root calcium concentration would influence both root tissue density (RTD) and root longevity, as calcium is important in stability of cell membranes (22), thus causing correlations between root persistence and root construction (5). Also, genes determining root cortical layers can influence not

Significance

Plants have evolved diverse root form and function across the Earth’s environment, yet only certain types of trait combinations have proved evolutionarily viable. Using a genetic mapping population of the native perennial switchgrass, our study demonstrates multiple genetic linkages among root morphology, growth, and turnover. Switching alleles derived from southern-origin ecotypes to alleles from northern-origin ecotypes increases root turnover but reduces tissue investment in root length construction. The genetic trade-off between construction and turnover likely facilitates the local adaptation of root strategy along the warm to cold climatic gradients of the species range. In practice, our study provides the genetic evidence that increasing switchgrass yield for bioenergy does not directly conflict with enhancing its root-derived carbon sequestration.

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only root thickness but also mycorrhizal colonization intensity (23), and intense mycorrhizal colonization is expected to reduce root mortality through defense against pathogens (24). Testing the heritability of root traits and genetic correlations of multiple root traits can be a sufficient approach to evaluate genetic causes of the RES, and comparing the quantitative trait loci (QTL) of the genetically correlated traits may reveal whether the trait relations are caused by selection on major pleiotropic genes or alleles at separate genes associated through linkage disequilibrium (19).

Understanding the genetic cause of RES is also critical for breeding plants with a root system with the potential to improve both ecological and economic values. Yet the variation of root traits and constraints on trait correlations is often an underexploited resource in plant genetic studies. Previous quantitative genetic studies of root traits and trait correlations have largely focused on populations of annual crop plants or the model plant *Arabidopsis* (25, 26), which might represent a narrow range of root strategies, given their relatively limited root morphological variation (27). Root functions of perennial plants are often more hierarchical across the root-branching system, such that the distal portions of the root system (i.e., absorptive roots) are often responsible for nutrient acquisition and frequently turnover as ephemeral modules, whereas the basal portions of the root system (i.e., transport roots) are often responsible for water and nutrient transport and persist for a relatively long period (28–30). The length ratios of absorptive to transport roots may decrease from growing season to nongrowing season for some perennial species (31, 32), and rapid root turnover of absorptive roots often indicates an acquisitive strategy (7). Thus, the genetic basis of the construction economics of both absorptive and transport roots, as well as their genetic correlations to various root functions, are then critical to understanding the intrinsic regulations of various plant belowground processes.

We examined the genetic basis of root traits and trait correlations of both absorptive and transport roots in perennial C₄ switchgrass (*Panicum virgatum* L.), which is a native grass widely distributed in North America and may have evolved diverse, adaptive strategies to the broad ranges of climatic and edaphic conditions (33). Our experiment utilized outbred progeny that recombined the genetic variation of four genotypes that are derived from lowland and upland ecotypes [SI Appendix, Fig. S1 (34)]. The northern-origin upland ecotypes are cold tolerant with smaller plant size, whereas southern-origin lowland ecotypes are more adapted to riparian habitats and exhibit erect growth. The larger canopy of southern lowland ecotypes often couples with a larger, denser, and thicker root system than upland ecotypes, which may result in positive feedback to shoot growth (14, 35, 36). Yet belowground traits are relatively less examined in switchgrass, and the adaptive significance of belowground traits and their role in ecotype formation has often been ignored. Also, whether the root morphological variations among switchgrass ecotypes can translate into root functional diversity, such as resource acquisition and root turnover remain largely unknown (5, 6).

Here, we measured several root functions that are relevant to the RES in our experimental plantings that included the grandparents and 384 outbred progeny of switchgrass. First, the productivity of absorptive roots (in terms of root biomass and root length for a given soil volume) largely indicates a plant's nutrient acquisitiveness, especially when competing with neighboring plants (37). Furthermore, since nutrients are often patchily distributed in soil, the precise placement of absorptive roots within the nutrient-rich patch relative to nutrient-poor soil is likely to determine the efficiency of plant nutrient acquisition [nutrient foraging precision (6, 38)]. The extent of root plasticity could be determined by genetic components and their response to environmental factors, since one ecotype of a plant

species may be able to increase root growth rate in response to a certain stimulus, whereas another ecotype may lack this characteristic (39, 40). Finally, we compare the length ratios of absorptive with transport roots in winter versus growing season to estimate the percentage of absorptive roots that persisted and turnover in the unfavorable season. We aim to address the following major question: Is the RES genetically supported?

We also estimate the genetic correlations between above- and belowground traits to understand the genetic basis of “whole-plant” trait coordination (41, 42). Greater shoot biomass often requires a larger root system, but there could be an allocation trade-off between roots and shoots, and plants in cold or dry climates tend to distribute relatively more resources to roots to enhance resource uptake than plants in warm or humid zones (43). In practice, the aboveground yield of switchgrass is indicative of potential utility as a bioenergy crop, and the belowground root residue is expected to increase soil carbon storage (44). However, the breeding of switchgrass for high aboveground biomass may not concomitantly result in a greater belowground biomass that leads to an increase in carbon sequestration. Clarifying how different genetic loci contribute to above- and belowground biomass productivity and turnover, as well as their genetic linkages to simple root morphological traits, presents economic and ecological opportunities to maximize yield of carbon-neutral products, while simultaneously increasing soil carbon sequestration, a win-win solution for mitigating the increasing atmospheric carbon dioxide.

Results

Trait Variation and Heritability. Switchgrass grandparents at the planting site differed substantially in their above- and belowground forms between the upland and lowland ecotypes. In the aboveground traits, lowland ecotypes were taller, flowered later, and yielded more shoot biomass than the upland ecotypes (Table 1). In the belowground traits, lowland ecotypes produced a higher density of root biomass and length than upland ecotypes (Table 1). Specific root length (SRL) of both absorptive and transport roots were smaller for lowland than upland ecotypes. Greater root diameters of lowland compared with upland ecotypes were only detected in the transport roots (third and higher orders) and root tips (first order) but not the absorptive roots (first and second orders) (Table 1).

Traits that had large variation between lowland and upland ecotypes showed relatively high broad-sense heritability (H^2), which was generally higher for aboveground traits (range 0.16 to 0.59) than root traits (range 0.03 to 0.42) (Table 1). However, the relatively low heritability of root traits did not necessarily result from limited genetic variation. Indeed, we observed comparable genetic coefficient of variation ($CV_g\%$) among above- and belowground biomass traits. The relatively low H^2 of root traits are primarily driven by the greater environmental coefficient of variation ($CV_e\%$) than the corresponding $CV_g\%$ (Fig. 1). Within the root system, we found that transport roots were more heritable in root diameter and SRL than absorptive roots, whereas absorptive roots were more heritable in the density of root length and biomass than transport roots (Table 1).

The percentage of absorptive root length decreased markedly from the growing season roots to overwintering roots (SI Appendix, Fig. S2), suggesting that absorptive roots of switchgrass were the root modules that were frequently deciduous compared with transport roots during the unfavorable season. Interestingly, although root turnover did not show much difference between the lowland and upland grandparents, we detected considerable heritability in traits related to root turnover, including the percentage of absorptive root length that persisted over the winter ($H^2 = 0.25$), the relative changes in the percentage of absorptive root length between growing season

Table 1. Aboveground and belowground traits of switchgrass ecotypes planted at Perkins, OK

	Lowland	Upland	<i>P</i> value	<i>H</i> ²	CV _g %	CV _e %
Aboveground						
Plant height (cm)	210.2	97.1	<0.001	0.47 ± 0.07	10.0	10.6
Shoot biomass (kg · plant ⁻¹)	5.7	0.3	<0.001	0.59 ± 0.06	45.9	38.1
Green-up time (Julian date)	81	80	0.790	0.16 ± 0.07	2.6	6.0
Flowering time (Julian date)	229	169	<0.001	0.54 ± 0.07	6.9	6.4
Belowground						
Transport roots						
Diameter (mm)	1.66	1.31	<0.001	0.31 ± 0.08	21.7	32.3
SRL (m · g ⁻¹)	2.0	3.6	0.001	0.20 ± 0.07	10.9	21.8
Tissue density (g · cm ⁻³)	0.26	0.25	0.89	0.03 ± 0.06	3.6	20.5
Length density (cm · cm ⁻³)	1.09	0.76	0.09	0.19 ± 0.07	5.7	11.7
Biomass density (mg · cm ⁻³)	5.86	2.24	<0.001	0.26 ± 0.08	50.3	84.3
Absorptive roots						
Tip thickness (mm)	0.19	0.17	0.02	0.13 ± 0.07	4.3	11.0
Diameter (mm)	0.83	0.80	0.64	0.08 ± 0.07	22.6	74.9
SRL (m · g ⁻¹)	33.6	48.2	0.04	0.17 ± 0.07	2.6	5.8
Tissue density (g · cm ⁻³)	0.06	0.05	0.22	0.14 ± 0.08	7.7	18.9
Length density (cm · cm ⁻³)	5.00	2.60	0.002	0.34 ± 0.08	6.5	9.1
Biomass density (mg · cm ⁻³)	1.55	0.60	<0.001	0.42 ± 0.08	25.0	29.4
Turnover						
Absorptive length% (growing season)	8	78	0.19	0.03 ± 0.06	1.5	8.4
Absorptive length% (overwintering)	55	49	0.25	0.25 ± 0.08	12.0	20.8
Relative root length turnover (%)	32	36	0.60	0.19 ± 0.08	19.3	40.2
Absolute root biomass turnover (mg · cm ⁻³)	0.49	0.42	0.76	0.09 ± 0.07	75.1	234.1
Nutrient foraging						
Root-foraging precision (%)	0.0	-27.9	0.17	0.05 ± 0.07	216.9	926.5

Trait values with significant difference between upland and lowland ecotypes are shown in bold. *H*² as well as CV_g% and CV_e% of each trait were also shown.

and winter (i.e., relative root length turnover, *H*² = 0.19), and the absolute change of absorptive root biomass between growing season and winter (i.e., absolute root biomass turnover, *H*² = 0.23). In contrast, the heritability for the percentage of absorptive root length during the growing season was very low (*H*² = 0.03). Limited heritability was also found in root-foraging precision, which was measured as the relative difference in absorptive root length

density between the nutrient-amended patch and unamended patch (*H*² = 0.05, Table 1 and Fig. 1).

Quantitative Trait Locus (QTL) Mapping and Genetic Effect. Overall, we detected 22 significant (*P* < 0.1 with 10,000 permutations) and 12 suggestive (0.1 < *P* < 0.25 with 10,000 permutations) QTL for the above- and belowground traits (Table 2). Among these QTL,

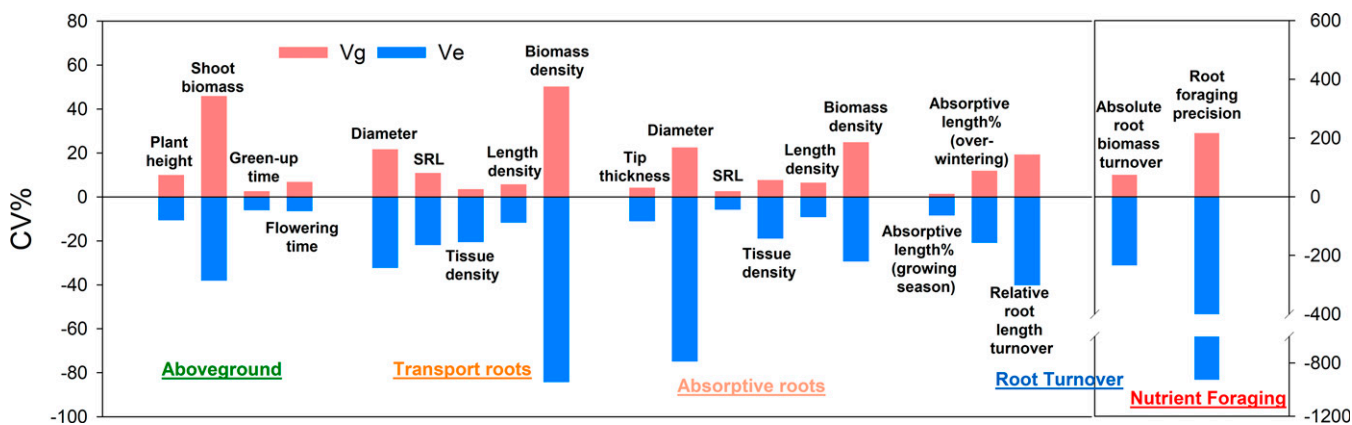


Fig. 1. CV_g% (red bars) and CV_e% (blue bars) of the aboveground and belowground traits. Because CV% were much larger for root-foraging precision and absolute root biomass turnover, the right y-axis represents the CV% of these two traits, and the left y-axis represents the CV% of other traits. Please see *Materials and Methods* for details of trait measurement and calculation. Note that green-up time was when new tillers sprouted from 50% of the crown area, and flowering time was when 50% of the tillers had panicles undergoing anthesis. Height was maximum height during the growing season. Roots were separated into absorptive roots (first and second orders) and transport roots (third and higher orders). SRL was the root length per mass, and RTD was the mass per turgid root volume. Tip thickness was the root tip diameter (first-order root) measured under stereomicroscope. Root length density and root biomass density were the length and biomass per soil volume. Absorptive length% was the length ratio of absorptive roots to total root length. Relative root length turnover is the relative changes in absorptive length% between growing season and nongrowing season. Absolute root biomass turnover is the absolute changes of absorptive root biomass between growing season and nongrowing season. Root-foraging precision was the difference in root length density in the two nutrient types (control versus nutrient amended) relative to averaged root length density, adjusted by potential unequal initial root length between the two nutrient types.

there were relatively more QTL per trait in aboveground than belowground, and the logarithm of the odds (LOD) scores were on average higher for aboveground QTL than belowground QTL (Fig. 2). In addition, there were no significant QTL detected for 7 out of 16 root traits but only one out of four aboveground traits. Among the root traits, root productivity (root length and biomass density) as well as root morphology (diameter, SRL, and tissue density) of both absorptive and transport roots were mainly influenced by multiple QTL at chromosomes 3N and 9N (Table 2 and Fig. 2). The percentage of absorptive roots persisting over the

winter and the relative root length turnover were mainly influenced by QTL at Chr6K@47.4. In addition, the percentage of absorptive roots persisting over the winter was influenced by a suggestive QTL at Chr3N@23.8. A QTL for absolute root biomass turnover was at Chr9N@50.2. No significant or suggestive QTL were detected for root-foraging precision (Table 2 and Fig. 2). The confidence intervals (CIs) ranged from 1.0 to 58.1 centimorgans (cM) for all significant QTL, with an average interval of 23.3 cM. For aboveground traits, each significant QTL on average spanned 20.7-cM distance and included 1,082 candidate genes.

Table 2. Significant and suggestive QTL for aboveground and belowground traits and principal components throughout the chromosomes

Trait	Chr	LOD	POS	CI_L	CI_H	Marker	Candidate genes count	Effect L1 × U1, %	Effect L2 × U2, %
Aboveground									
1* Flowering time	2K	8.76	0	0	1.0	Chr02K_1.973728	94	4.7	2.4
2* Plant height	2K	4.92	2.6	0	4.6	Chr02K_3.850984	281	7.3	1.6
3 Shoot biomass	2N	3.85	70.7	25.6	82.4	Chr02N_58.072303	2,891	17.5	1.0
4 Plant height	2N	3.95	71.5	52.3	98.2	Chr02N_58.231549	2,439	3.8	3.6
5* Flowering time	2N	5.31	73.5	69.6	100.1	Chr02N_59.529574	1,258	2.8	2.7
6* Shoot biomass	3K	5.50	33.2	27.2	85.3	Chr03K_15.525109	3,370	19.0	23.8
7* Plant height	3K	5.56	39.2	27.0	43.4	Chr03K_18.563419	1,083	4.5	5.4
8* Shoot biomass	3N	9.39	74.6	24.1	76.4	Chr03N_41.871736	2,647	12.8	44.5
9* Flowering time	5N	15.3	84.7	83.8	85.4	Chr05N_64.427563	100	7.1	0.2
10* Plant height	5N	14.3	85.1	84.0	85.8	Chr05N_64.620019	112	11.7	-2.1
11* Shoot biomass	7K	5.16	12.7	8.4	22.0	Chr07K_31.595266	766	24.8	-1.5
12* Shoot biomass	8N	4.55	27.6	21.9	39.8	Chr08N_38.138851	880	28.3	1.2
13* Flowering time	9K	4.99	23.3	8.3	38.6	Chr09K_7.425352	1,308	5.6	-1.2
14 Plant height	9K	3.65	35.8	4.0	55.6	Chr09K_12.132991	2,594	6.6	1.3
15 Plant height	9N	3.86	15.5	11.6	31.0	Chr09N_5.569642	1,025	3.9	1.4
16 Shoot biomass	9N	3.89	21.7	12.6	34.4	Chr09N_9.179245	1,176	21.7	9.1
Belowground									
1 Absorptive length% (growing season)	2N	3.96	30.7	22.8	56.7	Chr02N_15.898276	1,745	3.2	2.3
2 Absorptive length% (overwintering)	3N	3.88	23.8	14.3	84.0	Chr03N_6.675778	2,864	-2.1	7.6
3* SRL (absorp.)	3N	4.88	34.6	32.2	48.7	Chr03N_8.943292	933	1.3	-10.0
4 SRL (transp.)	3N	3.77	74.0	24.9	102.9	Chr03N_41.758701	3,414	-1.5	-12.9
5* Biomass density (absorp.)	3N	4.86	76.8	74.0	79.3	Chr03N_43.790867	79	-6.7	31.5
6 Length density (absorp.)	3N	4.14	76.8	74.9	80.6	Chr03N_43.790867	113	-9.3	24.4
7* Absorptive length% (overwintering)	6K	6.19	47.4	6.2	49.5	Chr06K_44.753959	2,609	9.0	10.3
8* Relative root length turnover	6K	5.90	47.4	6.2	49.8	Chr06K_44.753959	2,615	-15.6	-17.5
9 SRL (absorp.)	7K	4.07	22.0	20.0	35.7	Chr07K_36.952768	1,114	-8.9	4.3
10* Diameter (transp.)	9K	4.50	57.4	52.0	60.7	Chr09K_30.837754	965	2.7	4.4
11* Length density (transp.)	9N	4.18	48.2	46.2	66.6	Chr09N_23.825746	1,201	16.7	-15.7
12* Biomass density (absorp.)	9N	4.34	48.4	35.2	66.0	Chr09N_23.902939	1,644	22.9	-17.7
13* Absolute root biomass turnover	9N	5.39	50.2	45.1	54.4	Chr09N_25.62022	408	13.7	-16.6
14* Length density (absorp.)	9N	4.52	61.4	47.7	66.0	Chr09N_44.733142	1,101	24.4	-13.9
15* Biomass density (transp.)	9N	4.16	61.4	47.4	66.6	Chr09N_44.733142	1,139	18.7	-18.7
16* Diameter (transp.)	9N	4.19	83.3	32.8	100.8	Chr09N_64.285975	3,877	5.0	1.1
17 Diameter (absorp.)	9N	3.94	113.4	106.0	118.6	Chr09N_79.912252	339	-8.6	-2.2
18 RTD (absorp.)	9N	4.11	113.4	93.0	120.0	Chr09N_79.912252	1,094	22.1	8.3
Principal components									
1* PC1	2K	4.68	4.6	0.2	16.7	Chr02K_4.93201			
2* PC3	2N	5.61	48.4	34.3	71.5	Chr02N_39.466198			
3* PC1	3N	5.17	75.7	73.5	78.1	Chr03N_42.234697			
4 PC3	4K	3.66	2.9	1.3	4.2	Chr04K_2.836135			
5* PC3	5N	6.34	79.0	74.7	85.8	Chr05N_59.905171			
6* PC2	6K	4.52	44.9	3.2	49.0	Chr06K_44.356614			
7 PC1	6N	3.65	24.7	18.9	62.8	Chr06N_14.08987			
8 PC1	9N	3.74	61.4	42.6	64.4	Chr09N_44.733142			

The significance was tested by comparing LOD with thresholds of 10,000 permutations at an α of 0.10 (significant QTL) and 0.25 (suggestive QTL). QTL effect (percent) was calculated as the relative change of trait values when upland alleles are switched to lowland alleles in one of the two crosses. L1 = AP13, L2 = WBC, U1 = DAC, and U2 = VS16. See [Materials and Methods](#) and Fig. 1 for details of trait measurement and calculation. Statistical summary of the PCA is shown in [SI Appendix, Table S2](#). The annotations of candidate genes of each QTL are provided in [Dataset 1](#).

*Significant QTL.

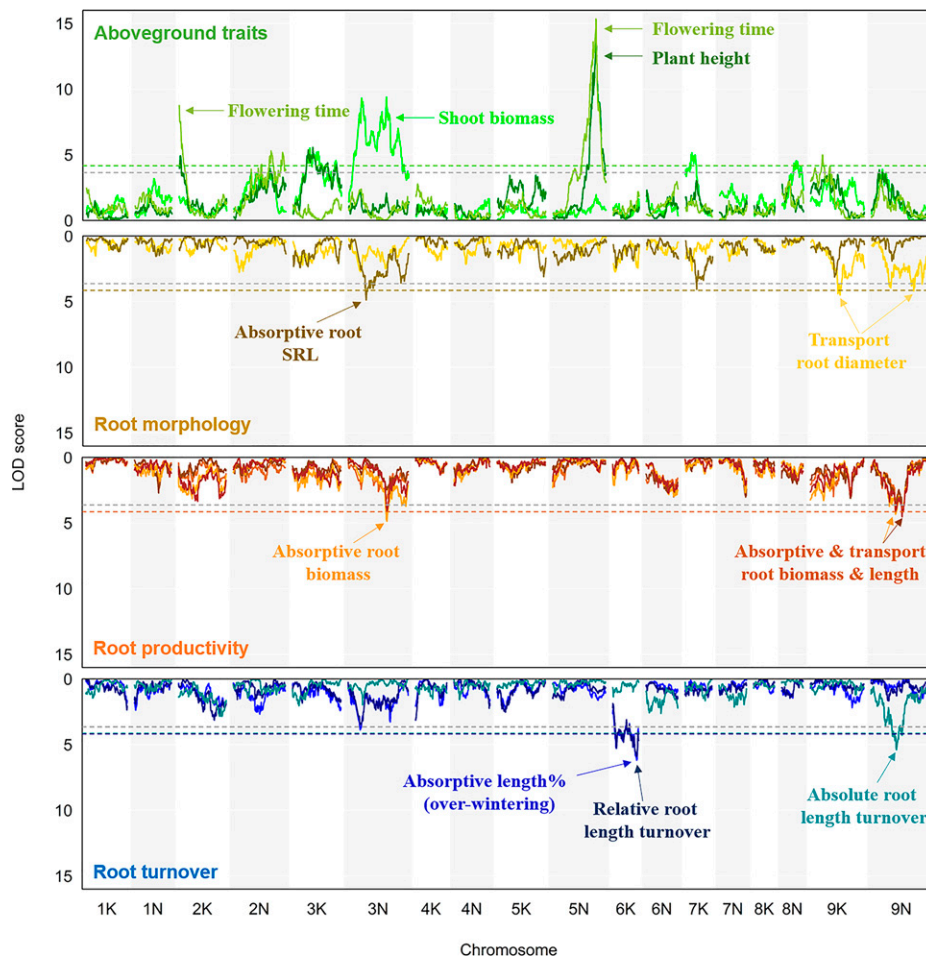


Fig. 2. Mapping positions of QTL across the aboveground and belowground traits. Only the traits with significant QTL are shown. The mapping position is the x-axis, and LOD score is the y-axis. LOD score is a measure of the strength of evidence for the presence of a QTL at a particular location. Significance thresholds of LOD score were calculated based on 10,000 permutations with an $\alpha = 0.1$ and represented by colored, short-dashed lines. The suggestive threshold was also calculated ($\alpha = 0.25$) and represented by gray, long-dashed lines. See *Materials and Methods* and Fig. 1 for details of trait measurement and calculation.

The intervals of belowground traits were on average wider (mean = 25.8 cM) and included more candidate genes (mean = 1,506) than those of aboveground traits (Table 2).

The effects of significant QTL were mainly in the direction of lowland versus upland ecotypic divergence for aboveground traits, root morphological traits, and relative root turnover traits (Fig. 3). For these traits, switching the upland alleles to lowland alleles at one QTL on average delayed flowering time by 3.6%, increased maximum plant height by 4.7%, increased shoot biomass by 19.1%, decreased absorptive SRL by 4.4%, increased the diameter of transport roots by 3.3%, decreased relative root length turnover by 16.5%, or increased the percentage of absorptive root persistence in winter by 9.6%. In contrast, for root productivity traits, as well as absolute root turnover, the significant phenotypic difference was mainly between the two lowland/upland heterozygous genotypes (lowland1 [L1]/ upland2 [U2] and lowland2 [L2]/ upland1 [U1]). Switching the upland alleles to lowland alleles may either increase, decrease, or has insignificant influence on root productivity and absolute root biomass turnover, depending on the parents of the crosses (L1 \times U1 or L2 \times U2) (Fig. 3).

Across all studied traits, we identified three main principal components (eigenvalues > 2) in the principal component analysis (PCA) (*SI Appendix, Fig. S3 and Table S3*), and significant QTL were mapped for all of the three principal components.

The primary component, which explained 22.6% of the variation, was mainly associated with root productivity, absolute root turnover, SRL of transport roots, and shoot biomass, with significant QTL mapped at Chr2K@4.6 and Chr3N@75.7. The second component, which explained 13.8% of the variation, was mainly associated with root diameter, tissue density, and relative root turnover/persistence in winter, with a significant QTL mapped at Chr6K@44.9. The third component, which explained 10.6% of the variation, was mainly associated with plant height, flowering time, and also relative root length turnover/persistence in winter, with significant QTL mapped at Chr2N@48.4 and Chr5N@79.0 (*SI Appendix, Fig. S4*).

Genetic Correlations. A strong pattern of genetic correlation was found within the aboveground traits and within the belowground traits, as well as across the aboveground–belowground traits (Fig. 4 and *SI Appendix, Table S2*). Shoot biomass, plant height, and flowering time, but not green-up time, were positively genetically correlated with each other (mean genetic coefficient of correlation, $|r_g| = 0.79$). The morphological traits of both absorptive roots and transport roots were often intercorrelated (mean $|r_g| = 0.65$). However, tissue density of transport roots as well as the diameter of root tips and diameter of absorptive roots were independent of the trait correlation network (Fig. 4 and *SI Appendix, Table S2*). SRL of both absorptive

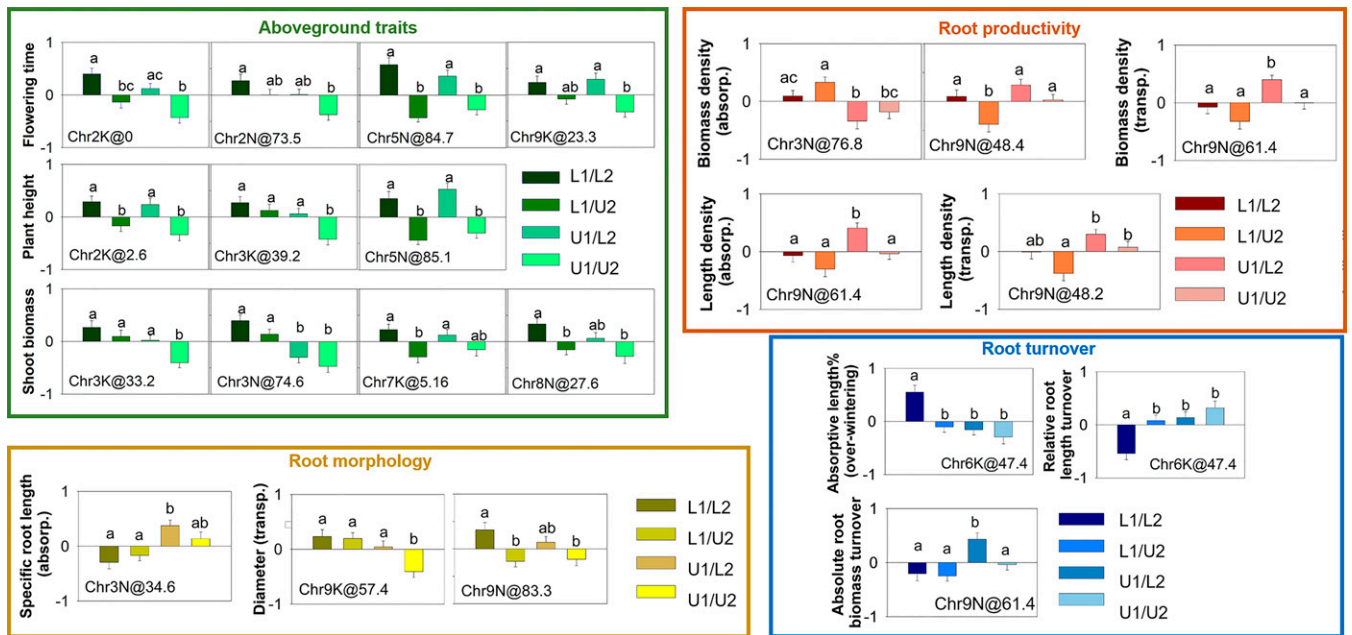


Fig. 3. The phenotypic effects of all significant QTL. Traits are grouped into aboveground traits (flowering time, plant height, and shoot biomass), root morphology (SRL of absorptive roots and diameter of transport roots), root productivity (biomass and length density of absorptive and transport roots), and root turnover (percentage of absorptive root length over the winter, relative turnover of absorptive roots, and absolute turnover of absorptive roots). In our outbred F_2 population, each individual has one of four combinations of F_0 alleles (L1/L2, L1/U2, U1/L2, and U1/U2; see *SI Appendix, Fig. S1* for details), and standardized phenotypic data were compared among the four allele combinations. Different letters indicate significant difference among the four allele combinations ($P < 0.05$). L1 = AP13, L2 = WBC, U1 = DAC, and U2 = VS16.

and transport roots were correlated with both root length and mass density (mean $|r_g| = 0.90$), all of which were also correlated with aboveground height and biomass (mean $|r_g| = 0.63$), framing a whole-plant, genetically interconnected network among root construction economics, root productivity, and aboveground size (Fig. 4 and *SI Appendix, Table S2*). For root turnover traits, we found that the percentage of absorptive roots that persisted over the winter was negatively correlated with absorptive SRL ($|r_g| = 0.74$), and the relative root length turnover was positively correlated with absorptive SRL ($|r_g| = 0.68$). Root-foraging precision was not genetically correlated with any other traits (Fig. 4 and *SI Appendix, Table S2*). The significant and suggestive QTL of these correlated aboveground–belowground traits were

clustered mainly at chromosomes 3N and 9N and less strongly at chromosomes 2N, 7K, and 9K (Fig. 2).

Discussion

Is the RES Genetically Supported? The generality of the RES remains one of the main knowledge gaps in belowground ecology (7, 15). In this study, we leveraged genetic mapping in divergent switchgrass ecotypes to examine the genetic basis of the RES. Our results provide evidence that RES is supported by genetic correlations between root construction economics and root tissue persistence/turnover (Fig. 4). Alleles that increase root persistence (or decrease relative root turnover) are not independent of

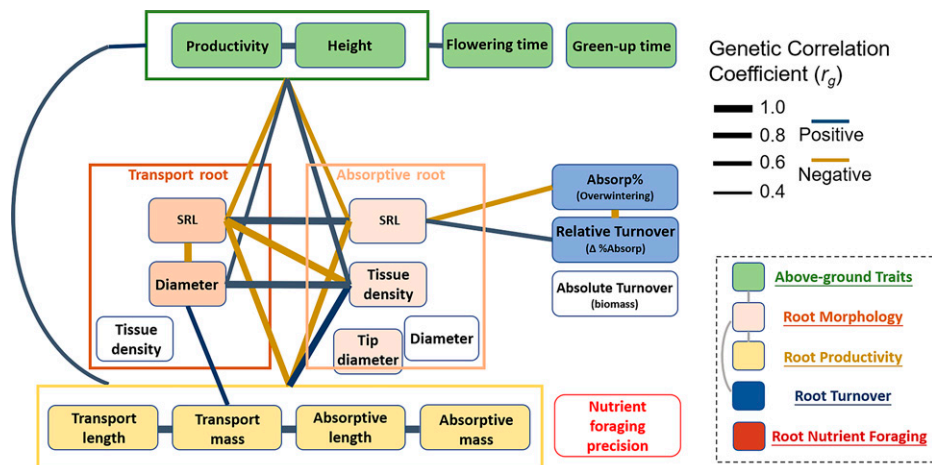


Fig. 4. Genetic linkages among the aboveground and belowground traits. Traits with low heritability ($H^2 < 0.1$) are shown in unfilled box. Significant correlations are shown in solid lines ($P < 0.05$). Blue lines are positive correlations, and yellow lines are negative correlations. Line thicknesses are proportional to the correlation coefficient. Correlations that are significant to a group of traits are linked by lines with averaged thickness connected to the box of the trait group. See *Materials and Methods* and Fig. 1 for details of trait measurement and calculation. See *SI Appendix, Table S2* for values of correlation coefficient.

alleles that decrease absorptive root SRL (or increase root biomass cost per length). Thus, only certain types of trait combinations tend to evolve in the switchgrass root system, such as ephemeral, high-SRL (inexpensive) roots and long-lived, low-SRL (costly) roots. Such phenotypic combinations can likely benefit plant competitive fitness (5, 17), and the genetic correlation structure would facilitate the evolution of these trait correlations.

Our results also suggest that a plant taxa's position in the RES is influenced by its locally adapted alleles. At a significant QTL that influences root senescence (Chr6K@47.4; Fig. 2 and Table 2), switching from lowland to upland alleles increases turnover of absorptive roots in winter by 16.6%. The upland deciduous root strategy is likely more adapted to the midlatitude region, where soil can freeze in winter and often experience drought (*SI Appendix, Table S1*). In contrast, the lowland overwintering root strategy is locally adapted to the low-latitude region, where winter climate is relatively mild. Such latitudinal patterns between lowland and upland alleles also exist in root construction economics (i.e., SRL). Switching from lowland to upland alleles at a significant QTL at Chr3N@34.6 increases absorptive root SRL by 4.4%. Previous observations in other plant lineages showed that plants tend to have greater absorptive SRL (i.e., lower-root construction cost) in temperate areas to cope with the higher drought frequency than in tropical biomes [*SI Appendix, Table S1* (27, 45)]. Interestingly, these two QTL are located at different chromosomes, suggesting that coselection on genetically independent QTL might have also facilitated trait correlations in the RES. Indeed, adaptive correlational selection on root persistence/turnover and SRL might have caused their genetic correlation to evolve (46). Moreover, because of the genetic linkages between these traits, selection on one trait (or both) would likely intrinsically cause evolution of the other trait, reinforcing the local adaptation of inexpensive, ephemeral, acquisitive root strategy versus costly, long-lived, conservative root strategy in different climatic zones.

Although there are significant genetic correlations between the key root traits in RES, the degree to which genetic correlations in the RES are due to pleiotropy or linkage disequilibrium is still uncertain (19). A meta-analysis summarized that 70% of significantly genetically correlated trait pairs showed shared QTL (47). Except for the significant QTL at Chr6K@47.4, there is a suggestive QTL of root persistence at Chr3N@23.8 that overlaps (although not very tightly) with the significant QTL of absorptive SRL (Chr3N@34.6) (Fig. 2), and the QTL effects are in the same direction of their genetic correlations in one of the two crosses (Table 2). Yet explaining the function of each QTL is challenging because there are often hundreds to thousands of candidate genes within the QTL intervals (Table 2). It is likely that, within the intervals of the overlapping QTL, there could be one or several key genes that regulate root-defensive chemistry, physiology, and/or anatomy, which subsequently influence both root construction economics and root persistence (5, 22–24). In contrast, the QTL of root turnover at Chr6K@47.4 is genetically independent of other detected QTL, suggesting its role in programming absorptive root senescence through unique signaling pathways (48, 49). The senescence programming of absorptive roots in the upland ecotype might also couple with their rhizome dormancy that benefits winter survivorship in northern latitudes (50).

In addition, while we have included suggestive QTL of root turnover by lowering the significance threshold, there could still be biologically important QTL with statistically low-LOD score that have been undetected during mapping. The polygenic structure of root turnover is expected because root longevity can be both endogenously controlled through phenological programming but also exogenously influenced by environmental and pathologic stresses (48, 49). The genetic correlations detected between SRL and relative root turnover/persistence are likely

the sum effects of a number of overlapping QTL. Because of low heritability in root traits and therefore low mapping power, undetected QTL could be frequent. Yet the limited heritability of root traits does not mean that they do not respond to natural or artificial selection. Indeed, there is considerable genetic variation in root traits, and the $CV_g\%$ of belowground traits are often comparable to those of aboveground traits (Fig. 1), indicating the importance of genetic influence on belowground traits. The limited heritability of root traits mainly results from relatively stronger environmental heterogeneity and greater difficulty in accurately measuring belowground traits than aboveground traits. Efforts to develop advanced techniques with high detecting efficiency and measurement accuracy of both root form and function in a large volume of soil are critical to improving root-based quantitative genetic studies.

It is also worth noting that genetic correlation does not always lead to phenotypic correlation. Among the F_2 progenies, absorptive SRL was not phenotypically correlated with relative absorptive root persistence/turnover in winter ($r_p = 0.01$, $P = 0.80$ for persistence and $r_p = 0.03$, $P = 0.66$ for turnover; *SI Appendix, Fig. S3*). This inconsistency between phenotypic and genotypic correlation indicates that genetic correlation can be largely masked by the environmental noise, which is in line with the relatively low heritability in these root traits. The large environmental variation or noise has probably led to the frequent controversy of RES in previous root phenotypic studies. Nevertheless, we argue that, in our study, we have functionally separated lateral absorptive roots from transport roots in more than 1,500 soil cores, and the genetic correlations calculated based on this dataset should provide reliable genetic evidence that RES will tend to exist, at least in terms of root construction economics and root tissue turnover.

Inconsistent with our hypothesis, we did not detect correlation between root nutrient-foraging precision and other RES-relevant traits. Our fertilized ingrowth core experiments provided little evidence for nutrient-driven dynamic foraging. The nutrient heterogeneity created here might not be strong enough to cause detectable, root-foraging behavior in switchgrass roots (51). In addition, plants have coevolved with a diverse array of symbiotic microorganisms, which could confound the one-dimension linkage between root construction economics and root nutrient acquisition strategy (8). In particular, arbuscular mycorrhizal fungi might substitute for roots in nutrient acquisition (6, 52), and nitrogen-fixing bacteria could differentially influence the potential nitrogen fixation between the switchgrass ecotypes (53). Nutrient foraging might also trade-off between root-foraging extent and thoroughness (54), in which one type more widely and sparsely explore the soil whereas the other type produces a more compact root system limited to the soil surface (26). We did observe denser absorptive root systems in the lowland than upland ecotypes in the upper soil profile. However, upland and lowland ecotypes tend to exhibit a similar pattern of vertical root distribution up to 1 to 2 m (14), suggesting that the foraging extent–thoroughness trade-off unlikely exist between switchgrass ecotypes.

Our result also confirms that absorptive SRL can be the key morphological trait in linking root turnover/persistence and framing the basic structure of RES, since other root morphological traits, such as absorptive root diameter, tissue density, and tip thickness, was not genetically linked to root turnover/persistence. We also found that root diameter and tissue density are orthogonal to SRL in our PCA analysis (*SI Appendix, Fig. S3*). These two traits might be uncorrelated with RES for anatomical reasons, such that diameter and RTD are measuring turgid root volume that may include structures with neglectable cost of construction and turnover [e.g., aerenchyma (55)]. Also, a relatively limited range of variation in root tip thickness in our population (0.15 to 0.25 mm), compared with global

variation [0.15 to 1.0 mm (3)], may statistically hinder the detection of significant QTL and genetic correlations. The SRL might also correlate with root physiology (e.g., respiration and nutrient uptake), as parallel correlation has often been documented in leaves (i.e., specific leaf area–respiration–photosynthesis). Yet recent studies showed that SRL only weakly and infrequently correlated with root respiration and nutrient uptake (10, 56, 57), possibly because activities of root-associated microorganisms contributed to the measured respiration rate and nutrient uptake rate. Nonetheless, future quantitative genetic studies that include more root nutrient acquisition traits as well as their mycorrhizal dependence and nitrogen fixation rate, are needed for a more holistic understanding of the genetic basis of RES.

Aboveground–Belowground Linkages. In addition to the genetic evidence of belowground RES, the aboveground–belowground trait correlations are also strongly genetically supported. Taller and bigger plants are genetically linked to greater productivity in terms of root length and biomass density. In addition, the first principal component in the PCA analysis, with significant QTL mapped at Chr3N, likely represents this size-related dimension in the trait variation space (41). Positive correlation between above- and belowground size has also been found in other studies of *P. virgatum* (58), other *Panicum* species (*P. hallii*, ref. 42), and species of other plant lineages (59, 60), probably because above- and belowground growth are interdependent, and synergetic modifications of both root and leaf growth are critical for most plant species to successfully survive and reproduce. Shared key genes may cause the genetic correlation in growth vigor of whole-plant tissue, and the locally adapted alleles of both shoot and root size might have been selected based on the climatic and edaphic conditions of their original locations (*SI Appendix, Table S1*). Nevertheless, the allelic effects of the significant QTL often vary between the aboveground and belowground growth-related traits. Switching from upland to lowland alleles can increase aboveground growth in both crosses across the significant QTL. In contrast, lowland alleles increase belowground growth in only one of the crosses, and which cross exhibits this allelic effect depends on the specific QTL in question (AP13 × DAC at Chr9N and WBC × VS16 at Chr3N; Table 2). Therefore, the genetic regulation of belowground growth could be more complicated than the regulation of aboveground growth.

In general, larger and taller plants require greater conductance of water and uptake of nutrients, and our genetic correlation analyses show that the greater requirements by shoots are likely met by a denser root system for uptake and thicker root system for transport. These findings are consistent with the positive correlation between maximum plant height and axile root diameter across 19 perennial grass species (61). We've detected pleiotropy or tight linkage at Chr9N for these correlated traits (Figs. 2 and 4). Thus, our results suggest broad genetic regulations and possible biophysical constraints of whole-plant vessel development and hydraulic conductance. In addition, we found that nearly all the significant QTL of shoot traits mapped in this study were close to their counterparts mapped in the same population across 10 sites [Table 2 (34)], indicating that QTL detected in this study are reliable despite being based on a single site.

Aboveground–belowground plant growth regulation may also be coordinated in terms of tissue turnover (62). We did not measure leaf turnover directly but included flowering time in the genetic analyses, which may correlate with leaf lifespan (21) and demonstrates plants' phenological strategy. Flowering time was genetically decoupled with belowground root turnover (Fig. 3), indicating potentially different selection pressures above- and belowground (17). The reserves stored in rhizomes would also drive root dynamics in the seasonally changing soil environments (50). Moreover, neither relative nor absolute

root turnover was genetically correlated with aboveground productivity. Therefore, breeding switchgrass with high-biofuel yield is likely independent from breeding with strong carbon sequestration potential via root turnover.

Conclusions

In summary, our study reveals multiple genetic constraints on the evolution of RES and plant aboveground–belowground organization. There are strong genetic correlations among root morphology, root productivity, and plant size. High-cost absorptive and transport roots were genetically constrained to genotypes with large productivity in both above- and belowground structures. Also, absorptive root persistence in winter was negatively genetically correlated with its SRL, suggesting that trade-off between resource acquisition and conservation can be intrinsically facilitated by their genetic correlational structure. Switching lowland alleles to upland alleles at a root-persistence QTL decreases the percentage of overwintering absorptive roots, providing genetic evidence of conservative and acquisitive strategies putatively locally adapted from warmer to colder regions. In contrast, genetic linkage between the precision of root proliferation in nutrient hotspots and the RES was not detected. Since functional hierarchy (ephemeral absorptive roots versus long-lived transport roots) is common in the root system of perennial plants, the genetic support of the RES found in switchgrass can likely extend to other perennial herbaceous and woody plant species.

Materials and Methods

Experimental Planting Design. The details of the experimental planting were previously described (34, 63). Briefly, the genetic mapping population was produced by initial crosses between switchgrass genotypes AP13 (L1) × DAC (U1) and WBC (L2) × VS16 (U2). Climatic and edaphic variation in the original collection locations of the four grandparents were shown in *SI Appendix, Table S1*. The F₁ hybrids of each of the two crosses were then intercrossed reciprocally to produce the four-way, outbred mapping population with 384 F₂ progenies (*SI Appendix, Fig. S1*). The F₂ population, parents and grandparents, were clonally propagated and planted at Perkins (35.99115° N, 97.04649° W) in the summer of 2015. Plants were randomized into a honeycomb design, in which each plant had four nearest neighbors all located 1.56 m away from each other (*SI Appendix, Fig. S1*). The experimental design is unreplicated in that we have a single-spaced plant for each unique outbred progeny. To prevent edge effects, a row of plants of the lowland Alamo cultivar was planted at every edge position of the plot. At this site, the annual air temperature is 15.2 °C and annual precipitation is 1,346 mm in 2019. Soil type is sandy loam to loam (sand = 49%, silt = 32%, clay = 19%, and organic matter = 1.62%).

Above- and Belowground Trait Measurements. Four aboveground traits were assessed as previously described (34). During the 2018 growing season, tiller green-up time, flowering time, and maximum height were measured. Green-up time was recorded as the day of year when a plant had sprouted new tillers from 50% of the area of the crown, and flowering time was the date when 50% of the tillers had panicles undergoing anthesis. Height was measured from the base of the plant to the uppermost point of the canopy, and the maximum height during the growing season was used. In early January 2019, plants were cut, and dry shoot biomass of each plant was measured.

After aboveground tiller harvest, two soil cores of each plant were collected in the following 2 d, with a Giddings Soil Sampler mounted on a trailer. The soil cores (5.08-cm diameter, 15.24-cm depth) were extracted at two points on opposite sides on the edge of the crown of each plant (*SI Appendix, Fig. S1*). Soil cores were stored in coolers until transferred to –20 °C freezers. Right after soil cores were collected, root ingrowth containers were installed into the resulting holes. Ingrowth containers were made from a rigid plastic mesh tube with 4-mm aperture, 5.08-cm diameter, and 17.78-cm length (2.54 cm above the soil surface) (6). It is worth noting that we focused on roots in the top of the soil profile, and the form and function of surface roots studied here may not fully represent those of deep roots (up to 3 m) in switchgrass. Ingrowth containers were filled with root-free soil collected near the switchgrass planting on the same day. For each plant, one container was supplemented with 10 g oven-dried and ground (powdery) switchgrass tiller material (total [N] = 3.8 mg · g⁻¹) in the first one-third depth of the container, which created an organic layer about 5 cm deep to serve as organic nutrient patches,

and the other container contained only sieved soil with no amendment (SI Appendix, Fig. S1). The tillers that were used were from the border plants of the experimental site (Alamo cultivar, harvested in November 2018). This dry plant material can be considered as a slow-release, organic fertilizer that imitates naturally formed, patchy nutrients, and as such, plant responses in this study are not directly comparable to findings using mineral fertilizer amendment or organic fertilizer with other amendment levels. After a few weeks, all soil cores were topped off with sieved soil (if needed) to make a level surface with the soil surface and to minimize settling impacts. All ingrowth containers were harvested in November following the aboveground harvest in 2019 (SI Appendix, Fig. S1), and all roots were kept frozen until processing.

Roots from both the initial soil cores collected in January 2019, as well as from ingrowth cores harvested in November 2019, were fully thawed, washed, and cleaned. Roots outside the ingrowth containers were removed (SI Appendix, Fig. S1). Only a very small fraction of roots (<5% length on average) in the ingrowth containers were from weeds (determined by root morphology, architecture, tissue strength, and color). Also, roots that were black or shriveled up were considered dead. Both dead roots and nontarget roots were discarded in later root processing. About half of each root sample was selected for scanning on a desktop scanner. To ensure homogenous subsamples, all roots in one sample were randomly laid out in a large rectangle tray, and roots on one side were chosen for scanning. Before scanning, roots were separated into absorptive and transport roots. Based on the preliminary screening, there were usually three to four root orders in total in the switchgrass root system [stream-based root system (28)]. The distal first- and second-order roots were soft, whereas the third- and higher-order (usually \leq fourth) roots were rigid, suggesting larger portions of cortex in the first two order root tissues and greater secondary xylem development in the third- and higher-order root tissues. Thus, we considered the first two orders of roots to be absorptive roots, and the third- and higher-order roots are transport roots. This separation criterion was further supported by the frequent turnover of first- and second-order roots rather than third- or higher-order roots in winter (SI Appendix, Fig. S2). Absorptive and transport roots were scanned with a desktop scanner at 400 DPI and saved as 8-bit grayscale JPG file (Epson 12000XL, Epson America, Inc.). Scanned images were processed with WinRHIZO (Regent Instruments, Inc.) to determine the average root diameter and total root length. The debris removal filter was set to remove objects with a length/width ratio lower than four. Scanned roots, as well as those that were not scanned, were oven dried (65 °C for 72 h) and weighed separately. Based on root weight fraction between scanned and unscanned roots, the total root length and root dry weight in the soil cores were calculated. Root length and biomass density were calculated by dividing by the soil core volume (308.89 cm³). SRL was calculated as the ratio between root length and root weight. RTD was root mass divided by turgid tissue volume determined by WinRHIZO. For each plant, the values of morphological traits, as well as root productivity in terms of length and biomass density, were averaged between the amended and unamended cores. We also measured the diameter of root tips (first-order roots) with a stereomicroscope under 35 \times magnification (45), with 30 first-order root segments measured for each soil core.

The ratio between absorptive root length and transport root length was calculated and compared between roots collected in November (growing season) and January (overwintering). The relative change in length ratio of absorptive:transport roots between growing season and overwintering roots was defined as relative root turnover (percentage). On the other hand, absolute root turnover was the absolute changes in absorptive root biomass between growing season and overwintering. Root-foraging precision was calculated as the difference in root length density in the two nutrient types (unamended versus amended) relative to the averaged root length density of

each plant. We were also concerned that, by chance, the original root lengths may have been unequal for the amended and unamended treatments. To remove this potential initial bias, adjusted foraging-precision was calculated by subtracting the apparent "precision" of initial root placement in "to-be-amended cores" relative to those in the "to-be-unamended cores" (64).

Quantitative Genetic Analyses. Details of genotyping and genetic map construction were previously described (34). We estimated quantitative genetic variance (V_g) for the measured features within our full-sib family using marker-based, realized relationship matrices and linear-mixed models using the *mmer* function in the *sommer* package (65, 66) in R (2020). Because of potentially high correlation between the additive and dominance relationship matrices in a full-sib family, it was not feasible to cleanly partition additive from nonadditive components of variance (67). As such, our analyses based on the additive kinship matrix alone could be biased upwards by any dominance variance which occurs. We thus report our estimates from the additive kinship matrix as V_g and our heritabilities as H^2 , which was calculated as V_g/V_p , where V_p is the total phenotypic variance. Phenotypic data were log transformed if needed. In addition, the V_e was calculated as $V_e = V_p - V_g$, assuming epistasis is limited. $CV_g\%$ and $CV_e\%$ were also calculated as V_g or V_e divided by mean values of the trait. Genetic correlations (r_g) were estimated through an identical mixed-effects model as heritability, except that the response variable was a bivariate set of two different traits. Spatial location information of each progeny in the experimental site was also included in our genetic correlation model. Statistical significance of genetic correlation was tested with likelihood ratio test between a model with genetic covariance and a constrained model with no covariance.

We mapped QTL for phenotypic traits using *R/qtl2*, with kinship matrix included under the leave-one-chromosome-out model (68, 69). We also performed a PCA on centered covariance matrices with all F_2 progenies and mapped the QTL for the main principal components. We calculated LOD thresholds through 10,000 permutations at an α of 0.10 to detect significant QTL throughout the chromosomes. We also included QTL with $0.10 < \alpha < 0.25$ as suggestive QTL in this study. The 1.0 LOD drop interval was assigned to localize each significant and suggestive QTL. The genes located in the 1.0-LOD CIs around the detected QTL were considered as candidate genes. The gene annotation file for switchgrass was accessed on Joint Genome Institute Phytozome 13 website: <https://phytozome-next.jgi.doe.gov/> (last accessed in August 2020). To examine the allelic effects at each detected QTL, we compared the corresponding phenotypic traits among the F_2 individuals with different types of alleles combination at the locus, including AP13 and WBC (L1/L2), AP13 and VS16 (L1/U2), WBC and DAC (L2/U1), and DAC and VS16 (U1/U2) (SI Appendix, Fig. S1). Also, the QTL effect was calculated as the relative change of trait values when upland alleles were switched to lowland alleles in one of the two crosses. All statistics were performed using R (70). Data are expressed as mean \pm SE.

Data Availability. All study data are included in the article and/or supporting information.

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