Reduced frequency of lateral root branching improves N capture from low-N soils in maize

Ai Zhan¹,² and Jonathan P. Lynch²,*

¹ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China
² Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA

* To whom correspondence should be addressed. E-mail: jpl4@psu.edu

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Abstract

Suboptimal nitrogen (N) availability is a primary constraint for crop production in developing countries, while in developed countries, intensive N fertilization is a primary economic, energy, and environmental cost for crop production. We tested the hypothesis that under low-N conditions, maize (Zea mays) lines with few but long (FL) lateral roots would have greater axial root elongation, deeper rooting, and greater N acquisition than lines with many but short (MS) lateral roots. Maize recombinant inbred lines contrasting in lateral root number and length were grown with adequate and suboptimal N in greenhouse mesocosms and in the field in the USA and South Africa (SA). In low-N mesocosms, the FL phenotype had substantially reduced root respiration and greater rooting depth than the MS phenotype. In low-N fields in the USA and SA, the FL phenotype had greater rooting depth, shoot N content, leaf photosynthesis, and shoot biomass than the MS phenotype. The FL phenotype yielded 31.5% more than the MS phenotype under low N in the USA. Our results are consistent with the hypothesis that sparse but long lateral roots improve N capture from low-N soils. These results with maize probably pertain to other species. The FL lateral root phenotype merits consideration as a selection target for greater crop N efficiency.

Key words: Branching, frequency, lateral root, maize (Zea mays), nitrogen (N), respiration.

Introduction

Suboptimal nitrogen (N) availability is a primary limitation to plant growth in terrestrial ecosystems (Tilman et al., 2002). In poor countries, low-N availability is a principal, pervasive constraint to crop production and therefore food security and economic development, as most smallholder farmers have limited access to fertilizer (Azejee et al., 2006; Worku et al., 2007). In developed countries, intensive N fertilization sustains high yields, but N use is generally inefficient, with only 30–40% of total N applied actually being harvested in grain (Raun and Johnson, 1999). Much of the remaining applied N is lost as surface runoff, leached nitrate (NO₃⁻) (London, 2005), or gaseous losses (Beman et al., 2005), all of which pose environmental concerns (Stulen et al., 1998). It is estimated that a 1% increase in N utilization efficiency could save ~$1.1 billion annually (Kant et al., 2011). Therefore, improved N efficiency would afford multiple global benefits.

The majority of research to improve N efficiency has focused on shoot biomass and grain yield as selection criteria (e.g. Moll et al., 1982; Raun and Johnson, 1999; Raun et al., 2002), and opportunities in the longer term to improve N transport and assimilation (e.g. Andrews et al., 2004; Hirel et al., 2007; Shrawat et al., 2008; Kant et al., 2011). The relevance of root traits for efficient N capture has received less attention. Root architecture has key importance for nutrient and water acquisition by positioning root foraging in specific soil domains in time and space (Lynch, 1995, 2011). For example, the ‘topsoil foraging’ ideotype appears to be particularly important for genotypic adaptation to low-phosphorus (P) soils (Lynch, 2011; Lynch and Brown, 2001; Richardson...
Nitrate, the dominant form of N in most agricultural soils, is highly soluble and is therefore subject to leaching into deeper soil strata (Thorup-Kristensen et al., 2009). Root systems with rapid exploitation of deep soil would optimize N capture in most production environments (Lynch, 2013). The ‘Steep, cheap, and deep’ (SCD) root ideotype consists of architectural, anatomical, morphological, and physiological phenotypes that work together to improve the capture of water and N in leaching environments by accelerating subsoil exploration (Lynch, 2013). Our focus here is on lateral root branching and length. According to the SCD ideotype, the frequency and length of lateral roots is important for N capture (Lynch, 2013). In the context of a cost/benefit analysis (Zhu and Lynch, 2004; Lynch and Ho, 2005; Zhu et al., 2005), N would be captured more efficiently by phenotypes with fewer but longer laterals capable of exploring a greater volume of soil accessible via mass flow of water, and therefore nitrate, than would phenotypes with a greater number of short laterals of equivalent total length. On the other hand, sparse lateral branching should concentrate internal resources on axial elongation and thereby increase rooting depth, and should reduce competition for N among neighbouring lateral roots. This prediction is supported by results from the functional-structural plant model SimRoot (Postma et al., 2014). Results from SimRoot indicate that sparse, long laterals were are optimal for N acquisition by decreasing competition among lateral roots for nitrate and reducing metabolic costs for root construction and maintenance. By reducing competition among lateral roots, sparse, long laterals effectively increase N uptake per unit root length, and by decreasing the metabolic cost, sparse, long laterals permit internal reallocation of nutrients to axial elongation, which is particularly beneficial under conditions of low-N availability (Postma and Lynch, 2011).

Lateral roots emerge from axial roots from lateral root primordial that initiate from pericycle founder cells (Malamy and Benfey, 1997; De Smet, 2007; Nibau et al., 2008; Péret et al., 2009). The formation of lateral roots is a major determinant of root system architecture (Nibau et al., 2008; Pérez-Torres et al., 2008). Genotypic differences in lateral root number and length have been reported in different species (Fitter, 1996; Fitter et al., 2002; Zhu et al., 2005; Trachsel et al., 2011; Lynch, 2013; Postma et al., 2014). In maize, Trachsel et al. (2011) observed significant genotypic variation in the density of lateral root branching, ranging from no lateral roots to 20 roots cm⁻¹. Also, in many Liliaceae and Orchidaceae, root systems of plants vary greatly in their architecture, from unbranched to highly complex branching patterns (Fitter, 1996; Fitter et al., 2002). Although lateral branching is a heritable trait (Zhu et al., 2005) and genes affecting lateral branching have been identified in several species, including maize (Doebley et al., 1995) and rice (Takeda et al., 2003), several studies report that lateral root number and length can be modulated by external NO₃⁻ availability (Drew and Saker, 1975; Robinson, 1994; Zhang and Forde, 1998; Linkohr et al., 2002; Hodge, 2004). For instance, in barley, external NO₃⁻ supply increased numbers of lateral roots and increased rates of lateral root elongation (Drew and Saker, 1975). It is reported that the stimulation of lateral root elongation appears to be attributable to a signalling effect from the NO₃⁻ ion itself rather than to a downstream metabolite (Zhang and Forde, 1998; Zhang et al., 1999). In Arabidopsis, the localized stimulatory effect of external nitrite on lateral root growth depends on the expression of the MADS-box transcription factor ANR1, which acts downstream of the dual-affinity nitrate transporter NRT1.1; both of them act together as an NO₃⁻ sensor, promoting auxin transport (Zhang and Forde, 1998; Remans et al., 2006; Krouk et al., 2010; Gojon et al., 2011).

The formation of lateral roots increases the sink strength of the root system, promoting the development of greater root length and thereby greater nutrient and water acquisition (Postma et al., 2014). However, in Medicago truncatula, the highly branched root architecture genotype TR185 showed a permanently N-starved phenotype (Bourion et al., 2014). Results from the functional-structural plant model SimRoot indicate that the optimal lateral root-branching density for N capture is less than that for P capture in maize (Postma et al., 2014). This is mainly because P is poorly mobile and well buffered in soil: most P is acquired within 1 mm of the root surface. If root spacing is, on average, >2 mm, some soil will remain unexploited. In contrast, nitrate diffusion in soil is three or four orders of magnitude faster than phosphate diffusion, so roots 10 mm apart will probably compete for NO₃⁻ after ~1 day (Nye and Tinker, 1977). Therefore, the overlap of N-depletion zones around roots of the same plant effectively reduces nitrate uptake efficiency (Berntson, 1994; Fitter et al., 2002). In addition, following the economic paradigm of plant resource allocation (Bloom et al., 1985), root construction and maintenance requires metabolic investment, which can exceed 50% of daily photosynthesis (Lammers et al., 2002). Production of more lateral roots than are needed for N capture would divert carbon and other resources from other root classes, including axial roots (Borch et al., 1999; Miller et al., 2003; Lynch, 2007), potentially slowing axial root elongation into deep soil strata. This is especially important for the acquisition of mobile resources which can accumulate in deep soil strata, like water and nitrate (Lynch, 2013). The few/long (FL) lateral root phenotype is therefore an element of the SCD ideotype for efficient N capture because sparse lateral branching should conserve internal resources, reduce competition for N among neighbouring lateral roots, and explore a greater volume of soil than a many/short (MS) lateral root phenotype.

The overall objective of this research was to assess the utility of lateral root number and length for N acquisition in maize under N-limiting conditions. Specifically, we tested the hypothesis that reduced lateral root number and increased lateral root length are associated with decreased root respiration, greater rooting depth, enhanced N acquisition, and greater plant growth and yield under N limitation.

Materials and methods
Greenhouse mesocosm study
Plant materials, experimental design, and growth conditions Eighteen recombinant inbred lines (RILs) of maize (Zea mays L.) from the intermated B73 × Mo17 population (IBM; Supplementary Table S1) were obtained from Shawn Kaeppler (University of Wisconsin, Madison, WI, USA), originally supplied by Charles Stuber and Lynn Senior at North Carolina State University (Senior et al., 1996;
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Kaepler et al., 2000). The 18 RILs with contrasting lateral root
branching density and length were chosen according to previous
experiments (Trachsel et al., 2011, 2013): nine RILs with FL lateral
roots and nine with MS lateral roots. The greenhouse experiment
was a randomized complete block design with a 2 × 18 factorial
arrangement of treatments. The factors were two N levels: optimum
N (high N, 4.5 mM) and suboptimal N (low N, 0.3 mM), and 18 gen-
types. Four replicates were staggered seven days between replicates
with time of planting treated as a block effect.

Seeds of 18 genotypes were surface sterilized in 0.05% NaOCl for
15 min and inbubed for 24 h in aerated 1 mM CaSO4, then placed
in darkness at 28 ± 1°C for 2 days. Seedlings of similar size were
transplanted to mesocosms consisting of polyvinylchloride (PVC)
cylinders 15.7 cm in diameter and 155 cm in height. The cylinders
were lined inside with plastic sleeves made of 4 mil (0.116 mm) trans-
parent hi-density polyethylene film, which were used to facilitate
root sampling. The growth medium consisted of a mixture (volume
based) of 50% medium size (0.5–0.3 mm) commercial grade sand
(with time of planting treated as a block effect.

The shoot was severed at the
growth nodal roots was based on counts. All roots emerging belowground
were classified as crown roots.

The shoot was severed at the

Chlorophyll content and net photosynthesis rate Plants were harvested
after 6 weeks after transplanting. Two days before harvest, leaf chlorophyll
content (SPAD) and net photosynthesis rate (Pn) were measured. The
SPAD readings were measured by using a chlorophyll meter (SPAD
502, Konika Minolta Sensing Inc., Osaka, Japan). For each plant, the
SPAD reading was measured in upper, middle, and lower portions of the
third youngest fully expanded leaf, and the average of all values for
each plant is presented. Leaf gas exchange of the third youngest fully
expanded leaf was measured with a Licor-6400 Infrared Gas Analyser
(Li-Cor Biosciences, Lincoln, NE, USA) using a red-blue light at PAR
intensity of 1200 μmol photons m−2 s−1, constant CO2 concentration
of 400 ppm, and leaf temperature of 25°C. The relative humidity was 40%.

Root respiration Root respiration of axial and lateral roots was meas-
ured. Three 10 cm root segments from the third whorl of crown roots
were excised 15 cm from the base. Lateral roots of axial roots were
removed with a Teflon blade (Electron Microscopy Sciences, Hatfield,
PA, USA). Excised axial and lateral root samples were patted dry
and placed in a 40 ml custom chamber connected to the Li-6400 IRGA
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(PA, USA).
Probabilities of significance were used to test differences among treatments, and Tukey’s Honest Significant Difference method ($\alpha = 0.05$) was used to compare the means.

**Results**

**Lateral root branching and length**

In mesocosms, the lines displayed the expected phenotypes, with MS lines having greater lateral root-branching density of crown roots than FL lines, although no significant difference was found in seminal and primary roots (Fig. 1A). Low N did not
significantly decrease lateral root-branching density in either FL or MS lines. In the field experiments, MS lines had greater lateral root-branching density of crown roots than FL lines (Fig. 1B, C). Lateral root-branching density of crown, primary, and seminal roots was not influenced by N treatment in either RS or SA. In the mesocosms, under high-N conditions, the FL and MS lines had equivalent total lateral root length. Low N significantly decreased total lateral root length of all root classes (Fig. 2).

**Effects of lateral root-branching density on root growth**

Under low N in mesocosms, axial root length (ARL) of crown roots was significantly different between FL and MS lines, though no difference was found in primary and seminal roots (Fig. 3). In low-N conditions, crown, primary, and seminal

![Fig. 4. Correlation of axial root length with lateral root-branching density of crown roots (A), primary roots (B), and seminal roots (C) in GH mesocosms at 42 days after planting. Each point is the mean of four replicates of each genotype.](image)

![Fig. 5. Root respiration of axial and lateral roots per unit ARL of FL and MS lines under high-N (HN) and low-N (LN) conditions at 42 days after planting in GH mesocosms. The data shown are means of four replicates of the nine genotypes in each phenotypic class in either HN or LN ± SE. Different letters represent significant differences (P < 0.05) compared within each root class.](image)
roots of FL lines had ~38, 39, and 15% greater ARL, respectively, than MS lines. The ARL of crown and primary roots was negatively correlated with lateral root-branching density (Fig. 4A, B). N limitation reduced root respiration per unit ARL of axial roots by 50% and reduced that of lateral roots by 46% (Fig. 5). Root respiration of lateral roots per unit ARL indicated no significant difference between FL and MS lines in both high-N and low-N conditions (1.87 and 1.83 nmol CO₂ cm⁻² s⁻¹ of FL and MS lines in high N; 1.72 and 2.10 nmol CO₂ cm⁻² s⁻¹ of FL and MS lines in low N). Under low N, root respiration of lateral roots per unit ARL of FL lines was 67% less than that of MS lines. Lateral branching was positively correlated with respiration of lateral roots per unit ARL (Fig. 6B).

Low N decreased root length density in both RS and SA (Fig. 7A–D). Under low-N conditions, FL lines had greater root length density in deep soil layers than MS lines (Fig. 7C, D). This result is also evident from the D₉₅ data. Under low N in the field, FL lines had significantly greater D₉₅ than MS lines (Fig. 7C–D). FL lines had a D₉₅ value of 57.9 cm in RS and 36.9 cm in SA compared to 49.6 cm and 30.8 cm for MS lines. Negative relationships between D₉₅ and lateral root-branching density in crown roots, primary roots, and seminal roots were found in both RS and SA (Fig. 8A–C).

**Root and shoot growth**

N deficiency significantly decreased relative shoot dry weight (RSDW) compared with high-N plants in GH, RS, and SA (Fig. 9, Table 1). Under low N, FL lines had 75% more RSDW at 42 DAP in the GH, 46% more RSDW at flowering in RS, and 41% more RSDW at flowering in SA compared with MS lines (Fig. 9, Table 1). Low N reduced Pn by 38% in mesocosms and 32% in the field in the USA (Fig. 10).
A, Table 1). Under low N, compared with the MS lines, FL lines had 39% greater Pn in mesocosms and 33% greater Pn in the field in the USA. Under low N, FL lines had 45, 33, and 34% greater SPAD readings than MS lines in GH, RS, and SA, respectively (Fig. 10 B, Table 1). In the mesocosms, RSDW and N content were positively related with the length of crown root and primary root axes (Fig. 11A, B), while in RS and SA, RSDW and N content were positively related with D95 (Fig. 12). In the field in the RS, D95 was positively correlated with grain yield (Fig. 13).

**Discussion**

Our results support the hypothesis that the phenotype of FL lateral roots is superior to MS lateral roots under N limitation, as evidenced by decreased root respiration, greater rooting depth (ARL and D95), increased N uptake, greater photosynthesis (Pn), leaf greenness (SPAD), plant biomass, and reproductive output (Figs 3, 5, 7, 9–13). Under low N in mesocosms, FL lines had less root respiration, and greater rooting depth and shoot biomass than MS lines (Figs 3, 5 and 9). Under low N in field environments in the USA and SA, FL lines had greater rooting depth (D95), greater biomass accumulation and N content, and in the USA had substantially greater grain yield than MS lines (Figs 7, 9, 11–13).

For the analysis of the physiological function of lateral root branching and length, we used RILs, which are valuable in the analysis of phenotypic traits governed by multiple genes, such as lateral root branching in maize (Zhu and Lynch, 2004; Zhu *et al.*, 2005). The use of RILs ensured that the phenotypes we compared shared a common genetic background (i.e. descending from the same two parents), without

![Fig. 9. Relative shoot dry weight (% of shoot dry weight in high N) under high-N and low-N conditions at 42 days after planting in GH mesocosms in the field in RS and SA at anthesis.](image)

**Table 1. Summary of analysis of variance for physiological parameters as influenced by N treatment and phenotype**

| Effect | Pn       | SPAD     | SNC       | SDW       | Y        |
|--------|----------|----------|-----------|-----------|----------|
| GH     |          |          |           |           |          |
| N      | 147.93***| 535.35***| 386.38*** | 492.81*** | –        |
| P      | 1.87†    | 3.81***  | 3.26***   | 5.68***   | –        |
| N × P  | 1.43†    | 2.98***  | 3.36***   | 5.84***   | –        |
| RS     |          |          |           |           |          |
| N      | 90.04*** | 314.85***| 182.04*** | 199.25*** | 98.92*** |
| P      | 1.68†    | 10.36*** | 1.56†     | 3.44**    | 4.44***  |
| N × P  | 2.64*    | 2.95**   | 3.25**    | 9.60***   | 2.59*    |
| SA     |          |          |           |           |          |
| N      | –        | 335.68***| 386.38*** | 511.46*** | –        |
| P      | –        | 5.04***  | 3.26***   | 3.70***   | –        |
| N × P  | –        | 7.51***  | 3.36***   | 3.01**    | –        |

Results are shown for the GH, RS, and SA experiments. SNC, shoot N content; SDW, shoot dry weight; Y, yield; N, N treatment; P, phenotype. Associated F-values and probabilities are shown (†, P ≤ 0.1; *, P < 0.05; **, P < 0.001; ***, P < 0.0001).

![Fig. 10. Photosynthesis rate (A) and SPAD (B) readings under high-N (HN) and low-N (LN) conditions at 42 days after planting in GH mesocosms and at anthesis in the field in RS and SA. The data shown are means of four replicates of the nine (GH) and five (RS and SA) genotypes in each phenotype in either HN or LN ± SE. Different letters represent significant differences (P < 0.05) compared within each location.](image)
artificially induced mutations or transformation events (Zhu and Lynch, 2004; Zhu et al., 2005). Each RIL is a distinct genotype, and comparison of several RILs permits the analysis of a phenotype in distinct genomes, thereby reducing the risk of confounding effects from pleiotropy, epistasis, gene linkage, or other genetic interactions (Zhu and Lynch, 2004). The use of isogenic lines is not suited to the physiological analysis of phenotypic traits controlled by many genetic loci in unknown ways. In the present study, we combined results from three distinct environments, greenhouse mesocosms, and two field environments, which is noteworthy, since the field includes many environmental factors including soil biota, soil temperature gradients, soil physical constraints to root elongation, and other environmental variables that may affect results, whereas greenhouse mesocosms are more simplified environments allowing more detailed measurements.

The fact that our results from RILs in greenhouse mesocosms and two field sites are all in agreement suggests that potentially confounding factors of any given environment are not driving the results.

Root systems of plants vary greatly in their architecture, from unbranched (found in many Liliaceae and Orchidaceae) to highly complex branching patterns (Fitter, 1996; Fitter et al., 2002). In maize, Trachsel et al. (2011) observed significant genotypic variation in the density of lateral root branching, ranging from no lateral roots to 20 roots cm$^{-1}$. In the present study, significant genotypic variation in maize lateral root branching was also found in greenhouse mesocosms and two field sites, with MS lines having significantly greater lateral root number and less lateral root length than FL lines (Figs 1 and 2). Several studies have pointed out the existence of mechanisms that control lateral root emergence and elongation. Zhang and Hasenstein (1999) reported that lateral root initiation and elongation in *Lactuca sativa* resulted from a balance between the basipetal flux of a cytokinin-like inhibitor derived from the root apex and the acropetal transport of a shoot-derived auxin that promotes lateral root development. Two pathways of NO$_3^-$ regulation of root branching were identified, one stimulating root elongation, called the localized stimulatory effect, in which the external NO$_3^-$ ion acts as a signal rather than a nutrient; and the other inhibiting branching initiation, called the systemic inhibitory effect, which appears to be positively correlated with plant tissue N level and is assumed to involve a phloem-mediated signal from the shoot (Zhang et al., 1999; Zhang and Forde, 2000).

Following the economic paradigm of plant resource allocation (Bloom et al., 1985), root construction and maintenance requires metabolic investment. The more roots that are initiated, the more carbon and other resources that need to be invested in root growth and maintenance, which
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may impair the growth of shoots and other roots, and may limit reproduction (Lynch, 2007, 2014). In greenhouse mesocosms, MS lines had greater root respiration of axial and lateral roots per unit ARL than FL lines under low-N conditions (Fig. 5), and lateral root branching was positively related with axial and lateral root respiration (Fig. 6). These results are in agreement with previous studies in maize which reported that the enhanced proliferation of lateral branches was accompanied by increased respiration of axial roots (Granato and Raper, 1989). Reduced respiration of lateral roots in FL lines would allow more metabolic resources to be available for the elongation of axial roots. Indeed, we found that FL lines had deeper crown, primary, and seminal roots in greenhouse mesocosms (Fig. 3), and a negative relationship between lateral root branching and rooting depth in greenhouse mesocosms (Fig. 4). Similar results were obtained at the two field sites. Under low N, FL lines had deeper rooting than MS lines (Fig. 7), and rooting depth was negatively related with lateral root branching at both field sites (Fig. 8).

This has practical implications, since N applied early in the season as nitrate or as N forms that rapidly convert to nitrate are subject to leaching with precipitation. The rate of nitrate leaching can exceed the development of root growth in deep soil strata, which is a significant cause of low recovery of N fertilizer (Wiesler and Horst, 1993; Raun and Johnson, 1999; Cassman et al., 2002; Chen et al., 2011). Under such conditions, plants with deeper roots may be more adaptive by increasing N uptake from deep soil strata, thereby improving plant growth and decreasing nitrate leaching. Furthermore, greater lateral root branching places roots closer together, which increases competition for internal resources, and reduces N uptake per unit root length, as modelled by Postma et al. (2014).

Increased lateral root branching increased the total sink strength of the root system, but decreased the average growth rate of individual lateral roots due to internal resource limitations (Postma et al., 2014). This tradeoff between the number and average length of lateral roots is also evident in a large experimental data set by Pagès and Pellerin (1994). Greater lateral root branching might increase the rate at which a soil domain is depleted, and favour the uptake of immobile nutrients like P, since most P uptake by roots occurs <1 mm from the surface of a root (Nye and Tinker, 1977). This means that lateral roots spaced >2 mm apart leave gaps of unexploited soil. In contrast, nitrate diffusion in soil is three or four orders of magnitude faster than phosphate. Roots 10 mm apart will probably compete for NO₃⁻ after ~1 day (Nye and Tinker, 1977). Therefore, FL laterals capable of exploring larger volumes of soil with greater spatial dispersion among roots are better able to capture mobile resources such as nitrate. The tradeoff between lateral root branching and length suggests that plants may have a plasticity response to nitrate availability which would result in FL laterals on low-nitrate soils. These predicted plasticity responses correspond with observations also found in the modelling results by Postma et al. (2014), and in the literature from artificial systems (López-Bucio et al., 2003; Gruber et al., 2013).

Although this study focused on maize, we suggest that the phenotype of FL lateral roots would improve N capture in other species. The root system architecture of sorghum is similar to that of maize (Lynch, 2013), so the FL concept may be
fully applicable. Other graminaceous species like wheat, rice, barley, and oats have the same basic root structure as maize and may also benefit from this phenotype, although greater density of nodal roots in tillering species may change the relationship of lateral root-branching density and resource capture. Our results are entirely supportive of inclusion of reduced lateral root number as a component of the SCD ideotype (Lynch, 2013). The SCD ideotype applies to both water and N capture, since both of these soil resources are often localized in deep soil strata under limiting conditions. The utility of FL phenotypes for water capture under water stress deserves investigation.

Genotypic differences in lateral root number and length have been reported in maize (Zhu et al., 2005; Truchsel et al., 2011; Lynch, 2013; Postma et al., 2014), as we also report in this study. Previous studies indicated that lateral branching is a heritable trait (Zhu et al., 2005) and genes affecting lateral branching have been identified in several species, including maize (Doebley et al., 1995) and rice (Takeda et al., 2003), making lateral branching and length a feasible target for plant breeding. Our results are entirely consistent with the hypothesis that FL lateral root branching reduces root metabolic costs and increases rooting depth, leading to greater N acquisition from low-N soil. Therefore we suggest that lateral root number and length deserves consideration as a trait to improve the N efficiency of maize in breeding programmes.

Supplementary differences in lateral root number and length have been reported in maize (Zhu et al., 2005; Truchsel et al., 2011; Lynch, 2013; Postma et al., 2014), as we also report in this study. Previous studies indicated that lateral branching is a heritable trait (Zhu et al., 2005) and genes affecting lateral branching have been identified in several species, including maize (Doebley et al., 1995) and rice (Takeda et al., 2003), making lateral branching and length a feasible target for plant breeding. Our results are entirely consistent with the hypothesis that FL lateral root branching reduces root metabolic costs and increases rooting depth, leading to greater N acquisition from low-N soil. Therefore we suggest that lateral root number and length deserves consideration as a trait to improve the N efficiency of maize in breeding programmes.

Supplementary Table S1. List of 18 RILs selected from the IBM population.

Supplementary Figure S1. Phenotypic variation in lateral root branching density and length under low-N conditions from the SA experimental site.

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