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Modelling concept of lettuce breeding for nutrient efficiency

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Abstract Modern lettuce cultivars are bred for use under high levels of input of water and nutrients, and therefore less adapted to low-input or organic conditions in which nitrate availability varies over time and within the soil profile. To create robust cultivars it is necessary to assess which traits contribute to optimal resource capture and maximum resource use efficiency. We therefore revisited earlier published results on root growth, resource capture and resource use efficiency of lettuce exposed to localized drought and nitrate shortage in a pot experiment. Root growth in a soil profile with localized resource shortage depended on the resource that was in short supply. We conceptualized a model describing nitrogen uptake and use efficiency. We also investigated the genetic variation among 148 cultivars in resource capture over time and soil depth and in resource use efficiency in four (two locations × two planting dates) field experiments. Cultivars proved to be highly diverse in their ability to capture and use resources. This ability, however, was strongly affected by other sources of variance, stressing the need for an eco-physiological model capable of reducing the residual variance and improving the expression and evaluation of cultivar differences in relation to both resource capture and use efficiency in lettuce. We showed that genetic variation was best expressed under limiting conditions. To improve the conceptualized model further we identified issues requiring further analysis, e.g., the physiological reasons why certain cultivars are capable of quickly responding to changes in the environment to maintain optimal resource capture.

Keywords Drought stress · Modelling concept · Nitrogen use efficiency · Organic · Root growth · Resource capture

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Introduction

With increasing awareness of health benefits of vegetables, world-wide demand and supply of lettuce have risen tremendously since 1960, making it nowadays one of the leading vegetables in terms of crop value (Boriss and Brunke 2005). Lettuce breeding has focused on increasing yield of marketable head size, targeting leaf development, leaf shape, and head formation (Pua and Davey 2007). As vegetative growth in lettuce, a crop with a short cycle, strongly depends on availability of water and nitrogen (Ouzounidou et al. 2013), the abundant supply of these two resources in a sustainable way is crucial. Lettuce is

usually very responsive to growth-limiting factors but not always efficient in capturing all the resources available or converting them into harvestable produce (Zhang et al. 2008). Nitrogen shortage, even when only temporary, can limit lettuce growth as the physiological or morphological mechanisms compensating for an impaired resource uptake may require some time before being triggered (Mou et al. 2013).

The availability of water and nitrogen over time and space largely depends on variable soil factors (Curtin et al. 2006). The role of the soil biological, physical, and chemical characteristics in making nitrogen and water available is even more important in organic and low-input systems than in conventional systems (Nautiyal et al. 2010). Indeed in the former systems the release of nutrients provided by organic fertilizers relies on soil characteristics such as temperature, moisture content, pH, texture, etc. (Mele and Crowley 2008). Enhanced soil life and improved organic matter content buffer processes in the soil–water–plant interface of organically managed soils (Masciandro et al. 2013). Resource availability in soils under organic management can therefore be less rapidly and timely influenced than in conventional soils where mineral fertilization and the use of chemicals can have prompt effects on crop growth (Clark et al. 1999). Organically grown crops may consequently be more prone to temporary water or nutrient shortage which may easily lead to yield reduction (De Ponti et al. 2012). In lettuce, yields in low-input and organic systems are often lower than in conventional systems: for instance Leogrande et al. (2013) found that lettuce head weight (fresh matter) in fields fertilized organically can be 16–17 % lower compared to fields where mineral fertilization was applied.

One way to secure stable yields over a wide range of environmental conditions may be to breed robust lettuce cultivars (cf. Ceccarelli et al. 1991). Robustness is defined as the ability of the cultivar to perform well despite the presence of various environmental stressors (Kerbiriou et al. 2013a). Plasticity in morphological traits or physiological processes supporting continued nutrient capture, flexible internal storage and transport regimes, and improved nutrient use efficiency could create robustness, as such characteristics may enable the plants to withstand short periods of mild stress by conserving growth rates (Liao et al. 2001). In woody and herbaceous species, for instance, Mou et al. (2013) demonstrated that stable reduction in nutrient availability triggered morphological changes in root and shoot mass, and that physiological plasticity in nutrient foraging at the root level was less predictable, especially in temporally variable nutrient availability. In pot experiments with lettuce, Kerbiriou et al. (2013a) showed that the resource that was in short supply and the timing of the shortage determined the response.

In Europe, commercial lettuce cultivation entails the transplanting of seedlings grown in root blocks, consequently breaking the taproot; as a result, plants have a shallow root system mostly located in the top soil layers (0.0–0.2 m). Moreover, as lettuce breeding has mostly been focusing on improved head characteristics, root morphological and physiological traits have not been yet fully exploited.

Compared to modern commercial lettuce cultivars, wild lettuce species have a strong taproot (up to 0.5 m deep in the soil profile) (Johnson et al. 2000). This morphological feature enables wild lettuce species to cope with drought stress as they can extract water from deeper soil layers (Johnson et al. 2000). Breeding lettuce for improved root system architecture may then be one of the strategies to increase the capture in space and time of soil-bound resources, such as water and nitrogen.

In field experiments using four lettuce cultivars, Kerbiriou et al. (2013b) showed that larger root mass was in general positively associated with larger nitrogen capture throughout the soil profile, and that cultivars which had a larger root mass also had a larger shoot weight. On the other hand, a cultivar with a smaller root system but better nitrogen use efficiency than the other cultivars displayed stable yields across experiments, highlighting that the use of the resources captured below-ground is also important to secure stable yields across environments (Barlow 2010). Water and nitrogen capture and use efficiency are complex traits which are strongly affected by large genotype × environment (G × E) interactions (Jackson et al. 1996); their influence on crop performance and the genetic control of their expression can therefore be difficult to assess. Understanding the physiological mechanisms underlying water and nitrogen capture and use efficiency, as well as dissecting such traits into simpler, biologically meaningful component traits, is a major challenge which can be tackled by eco-physiological modelling approaches (Yin et al. 2004; Hammer et al. 2006; Yin and Struik 2008, 2010).

Because models can predict crop performance, account for G × E, and capture spatial and temporal dimensions of processes, they provide a valuable insight into the traits involved in diverse physiological and morphological mechanisms (Yin et al. 2004; Hammer et al. 2006; Yin and Struik 2008, 2010); models can thus be used for breeding purposes, by pointing out which traits are biologically relevant, less influenced by G × E and amenable for selection (Hammer et al. 2006; Postma et al. 2014). Models can also help to assess which markers account for the largest proportion in variance of a trait in a certain environment (Yin and Struik 2012; Gu et al. 2014). They can also test ideotypes, predict which environments will be very suitable for specific genotypes, and evaluate which genotypes are needed for specific environments (Yin and Struik 2012; Gu et al. 2014).

Several studies attempted to understand physiological mechanisms underlying responses to temporary or spatial limitations in water and nutrient supply (Kerbiriou et al. 2013a). To the best of our knowledge, no model is currently available that can include genetic information, physiological and morphological mechanisms involved in water and nitrogen capture and use efficiency above- and below-ground, their dynamics in space and time, and eventually, the influence of environmental conditions thereon. As a strategic decision tool, such a model would teach the breeder which trait should be targeted in the considered breeding environment.

However, the understanding of all G × E interactions and their integration into existing crop models is very tedious, requires a specific model design with strong heuristic power (Yin et al. 2004; Yin and Struik 2008) and requires numerous empirical and theoretical steps for proper calibration and validation. As a step towards the design of such a model, we propose in this study to:

(1) Investigate the physiological and morphological mechanisms involved in water and nitrogen capture and use efficiency in lettuce;
(2) Design a conceptual model based on these investigations;
(3) Assess the genetic variation in traits related to resource capture and use as indicated by in-depth phenotyping studies and the model;
(4) Assess the (relative) importance of G × E interactions.

In order to examine the elements above, we build on a previously published pot trial (Kerbiriou et al. 2013a) and four additional field experiments with a set of 148 commercial cultivars.

Materials and methods

Two types of experiments will be described. A pot experiment ('pot trial'), published by Kerbiriou et al. (2013a), which had been designed to observe the effects of localized nitrogen shortage or drought on lettuce shoot and root growth, was re-analysed. To investigate the role of below-ground morphological and physiological mechanisms involved in shoot performance, water and nitrogen capture, as well as root length and mass in each 0.10 m layer over a 0.40 m soil profile was measured during shoot growth. These measurements were related to nitrogen and water use efficiency calculated based on shoot measurements during growth.

A population of 148 commercial lettuce cultivars was phenotyped for resource capture and yield in four experiments, by planting them at two locations in the spring or summer season of two consecutive years ('field trials'). Water and nitrogen in each 0.10 m layer over a 0.40 m soil profile were measured during growth, and related to water and nitrogen use efficiency at harvest (based on marketable yield). These data have not been published before and therefore materials and methods of these trials will be described in detail in this paper.

Pot trial

The materials and methods used in this experiment are described in detail in Kerbiriou et al. (2013a). In brief, seeds of butterhead cultivars 'Pronto' and 'Matilda' were raised in a greenhouse and transplanted at the 5-leaf stage to PVC tubes of 0.2 m diameter and 0.4 m length. The tubes were placed in a fully conditioned greenhouse. Individual pots were weighed twice a week, and watered to bring pot weights back to the required level, while compensating for changes in plant fresh weight.

Treatments included various combinations of drought and nitrogen shortage in the upper and the lower pot compartment (cf. Table 1). Measurements were made 2, 4 and 6 weeks after transplanting in the

Table 1 Treatments applied in the pot trial for both cultivars (Source: Kerbiriou et al. 2013a)

		Treatments				
		Control	DST[a]	NST[b]	DST + NSB[c]	NST + DSB[d]
Upper compartment (0.00–0.20 m)	Fertilizer (g NO_3–N)	0.625	0.625	0.178	0.625	0.178
	Water status (v:v; %)	14	6	14	6	14
Lower compartment (0.20–0.40 m)	Fertilizer (g NO_3–N)	0.625	0.625	0.625	0.178	0.625
	Water status (v:v; %)	14	14	14	14	6

[a] Drought stress in top compartment

[b] Nitrogen stress in top compartment

[c] Drought stress in top compartment combined with nitrogen stress in bottom compartment

[d] Nitrogen stress in top compartment combined with drought stress in bottom compartment

greenhouse, corresponding to 288, 512 and 768 °Cd, respectively.

At each harvest, the content of each pot was divided into four layers of 0.1 m each. The roots in each layer were dried at 105 °C for 16 h for dry weight assessment. For each layer, a soil sample was taken to measure NO_3–N content using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA). NO_3–N uptake from a soil sample was calculated as the difference with the NO_3–N content in a soil sample taken from a pot without a plant. Data were analysed by a two-way ANOVA using Genstat 14th Edition (Hempstead, UK).

Field trials

The large-scale field trials using a population of 148 lettuce cultivars grown in four different environments enabled to assess the potential genetic variation existing in the physiological mechanisms regulating resource capture and use efficiency identified in the pot experiment and conceptualized in the model design. Hundred and forty eight commercial butterhead cultivars suitable for field spring/summer conditions were selected for this study.

Seeds used for the trials originated from seed lots produced under the same environmental conditions and were sown in 0.04 × 0.04 × 0.04 m organic peat blocks (Jongerius, Houten, the Netherlands) after breaking seed dormancy by exposure to 4 °C for 24 h. Transplants were raised in a greenhouse with day temperature of 20 °C and night temperature of 15 °C. Transplanting was done when the transplants had 5–7 leaves and few roots emerged out of the peat block. In the field, plant arrangement was 0.3 × 0.3 m.

Two field trials were carried out at each of two different locations: Wageningen (51.97° N, 5.67° E, The Netherlands) in spring and summer 2010, and Voorst (52.23° N, 6.08° E, The Netherlands) in spring and summer 2011 (see Table 2 for exact planting dates). Both locations had a uniform, sandy soil profile up to 0.5 m depth and adequate structure, but relatively low organic matter content and water retention capability. The sites had been cropped uniformly in the previous 5 years on a larger surface than the area covered by the trials. They were certified organic and managed according to organic standards during the experiments.

Each trial included two repetitions. The experimental set up was a complete randomized block design, each block consisting of 150 plots to which a cultivar was randomly assigned. Two plots per block were left empty for measurements in bare soil. A plot with plants consisted of 25 individuals (5 × 5 plants) of the same cultivar. Measurements were done on the nine inner plants.

All trial fields were uniform, certified organic and managed according to organic standards during the experiments. Fertilization was provided by applying 100 kg/ha nitrogen, from seaweed pellets (9 % N, 3 % P, 3 % K + 3 % MgO, EcoFertiel, EcoStyle, Appelscha, the Netherlands) on the day before transplanting. Irrigation was not provided.

For each trial, weather data (air temperature, radiation, rainfall) were recorded daily (Voorst) or hourly (Wageningen) at the nearest weather station. Cumulated degree-days (based on air temperatures), as well as cumulated rainfall at each sampling date for each trial, are shown in Table 2. Cumulated degree days at each sampling date were calculated as the sum,

Table 2 Transplanting- and final harvest dates, and weather conditions during the four field trials

Year	2010				2011			
Location	Wageningen				Voorst			
	Trial 1		Trial 2		Trial 1		Trial 2	
Planting date	20-05-2010		22-06-2010		22-03-2011		09-06-2011	
	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest
Sampling date	14-06-2010	05-07-2010	19-07-2010	28-07-2010	19-04-2011	17-05-2011	05-07-2011	25-07-2011
Cumulative rainfall (mm)	18	48	90	104	16	27	49	145
CDD* (°Cd)	357	793	607	782	174	481	329	590

* Cumulated degree-days (using 4 °C as base temperature)

between the date of transplanting and the sampling date, of the degrees above 4 °C (base temperature for lettuce), based on an average daily temperature.

Table 2 summarizes the characteristics of the contrasting environments in the four trials. During Trial 2, 2010 the environment was apparently the most conducive to lettuce growth; during this trial, the plants received about 800 °Cd and more than 100 mm rainfall (Table 2). In Trial 1, 2010 and Trial 1, 2011, conditions were relatively dry with only 48 and 27 mm cumulative rainfall received over the whole trial period, respectively. This poor rainfall was associated with relatively mild temperatures in the case of Trial 1, 2010, where the temperature sum reached a final value of 793 °Cd, but temperatures were lower during Trial 1, 2011, where temperature sum only reached a final value of 500 °Cd. Trial 2, 2011 had the wettest conditions, with 150 mm rainfall received during the trial period, but especially concentrated shortly before final harvest (25-07-2011).

Soil samples were taken every 0.1 m over a depth of 0.4 m outside of the peat block, using a 0.06 m diameter and 0.4 m long auger, during growth ('intermediate sampling') and at final harvest (cumulated degree days at the moment of sampling are detailed in Table 2). For three plants per plot, soil samples taken in each soil layer were pooled to account for plant-to-plant variation. Volumetric soil moisture content in each layer (soil [H_2O], v:v) was recorded after drying the sample at 40 °C for 48 h. Water left over the 0.4 m soil profile (mL) was calculated based on the soil [H_2O] measurement in each layer over a soil column of 0.1 m radius (R) and 0.4 m depth. Nitrate content (soil [NO_3], ppm) in each 0.1 m soil layer was measured using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA) using the method described previously by Sibley et al. (2009) and also used in Kerbiriou et al. (2013a). The total nitrate left over the 0.4 m soil profile (g) was calculated based on the nitrate concentration in each layer over a soil column of 0.1 m radius (R) and 0.4 m depth.

Shoot measurements were done only at final stage of the growth. Fresh weight and dry weight (g per plant) were assessed based on three plants per plot at final harvest, which took place 5–9 weeks after transplanting depending on trial. Plant [N] (g N g^{-1} DM) was measured using the Kjeldahl method, based on the grinded material of three plants per cultivar and per

replicate within a trial. Physiological Nitrogen Use Efficiency (NUE, g DM g^{-1} N in heads) was calculated based on the head [N]: NUE = 1/(head [N]). Plant N was calculated as average head dry weight × head [N]. Plant H_2O was calculated as plant fresh–plant dry weight. Plant [H_2O] (the amount of water per amount of dry matter) was calculated as plant H_2O/plant dry weight.

Data were statistically analysed by a one way ANOVA using the statistical package Genstat 15th Edition (Hempstead, UK). To calculate the variance components, we used the REML procedure in Genstat 15th Edition (Hempstead, UK) with the following model:

Genotype by (Year/Trial/Sampling) with all terms of the model as random terms.

This equals the following model for the soil measurements ([NO_3] in each 0.1 m layer of the 0.4 m soil profile and total NO_3 of the whole 0.4 m soil profile, and soil moisture content in each 0.1 m layer of the 0.4 m soil profile and the volume of water left over the whole 0.4 m soil profile):

$$\begin{aligned}\text{response} = {} & \text{var(genotype)} + \text{var(year)} \\ & + \text{var(trial within year)} \\ & + \text{var(sampling within trial within year)} \\ & + \text{var(genotype by trial within year)} \\ & + \text{var(genotype by sampling within trial within year)} + \text{var(residual)}\end{aligned}$$

For the shoot measurements, as they were made only at final harvest (plant fresh and dry weight, plant [N], plant N, plant NUE, plant [H_2O], plant H_2O), it equals to the model:

$$\begin{aligned}\text{response} = {} & \text{var(genotype)} + \text{var(year)} \\ & + \text{var(trial within year)} \\ & + \text{var(genotype by trial within year)} \\ & + \text{var(residual)}\end{aligned}$$

with response being the total variance observed for a variable, var(genotype) the proportion of the total variance due to the genotypic effect, var(year) the proportion of the total variance due to year effect (confounded with location as trials within a year were carried out at the same location), var(trial within year) the proportion of the total variance due to trial effect (each year counted two trials), var(sampling within trial year) the proportion of the total variance due to sampling effect (two sampling dates within a trial) and var(residual) the residual variance. The other variance components were variances associated with interactions. Block effects were not statistically significant and therefore block effect was not accounted for in the analyses to enhance model power.

Results and discussion

Assessing physiological mechanisms regarding resource capture and use

General physiological mechanisms regulating root growth and nitrogen capture and use efficiency in relation to shoot growth were assessed by carrying out the pot trial. This section focuses on the processes involved in spatial root growth and resource capture in the soil.

Spatial root proliferation is resource-specific

Both cultivars reacted very similarly to the treatments; mainly the results for 'Pronto' are presented in this section. Figure 1 shows the fraction of the total root mass present in each layer at different sampling dates for this cultivar. In the control treatment (Fig. 1C), on average 64 % of the total root mass was allocated to the upper compartment at the third sampling (768 °Cd). When drought was applied in the upper compartment, this fraction increased: on average 73 % of the total root mass was present in the upper compartment at the third sampling (768 °Cd) (Fig. 1A). When drought stress in the upper compartment was combined with nitrogen stress in the lower compartment (Fig. 1C) this fraction increased even more, with 77 % of the total root mass being allocated to the upper compartment. Only 23 % of the total root mass developed in the lower compartment, compared to 36 % for the control treatment.

The pattern was opposite when nitrogen stress was applied in the upper compartment: at the third sampling (768 °Cd), the fraction of roots allocated to the upper compartment was lower than in the control: 54 % for the 'NST' treatment (Fig. 1D); but in the lower compartment it was higher than in the control (46 % for the 'NST' treatment). This pattern was reinforced when nitrogen stress application in the upper layer was combined with drought stress in the

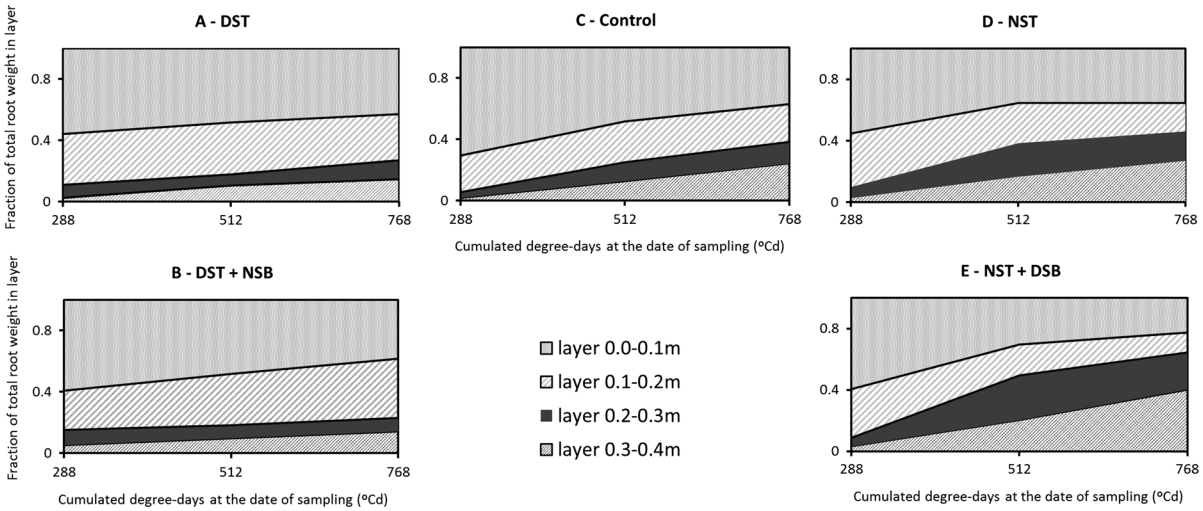

Fig. 1 Fraction of total root weight allocated to each layer (layer 0.0–0.1 m, layer 0.1–0.2 m, layer 0.2–0.3 m and layer 0.3–0.4 m) at different sampling moments during the trial (228, 512 and 768 °Cd) for each treatment (**A** drought applied in the *upper* compartment ('DST'), **B** drought stress applied in the *upper* compartment combined with nitrogen stress applied in the *lower* compartment ('DST + NSB'), **C** control treatment, **D** nitrogen stress applied in the *upper* compartment ('NST'), **E** nitrogen stress applied in the *upper* compartment combined with drought stress applied in the *lower* compartment ('NST + DSB') for the cultivar 'Pronto' in the pot experiment

lower compartment (Fig. 1E): the fraction of total root mass present in the upper compartment decreased to 36 % and the fraction of total root mass present in the lower compartment increased to 64 %.

Solely in dry soil additional root proliferation increases nitrate capture to a limited extent

Figure 2 shows the relationship between the root mass in a 0.1 m layer and the fraction of total nitrate present in the layer which was captured in the upper compartment (0.0–0.1 and 0.1–0.2 m layers) and in the lower compartment (0.2–0.3 and 0.3–0.4 m layers) in the five treatments (as 'Pronto' and 'Matilda' exhibited the same behaviour in this pot trial, data of these two cultivars were pooled together in the graphs). This figure shows that in this pot trial, a significant fraction of the total amount of nitrate available could be captured with little root mass: in the control treatment for instance less than 0.1 g of roots in either of the compartments were able to capture more than 40 % of the nitrate available in the soil layer (Fig. 2A, B). Moreover, this figure shows that roots kept growing in a layer although no more nitrate was available for uptake: this is clear in Fig. 2G where 100 % of the total amount of available nitrate in the layer was captured already between Sampling 1 (288 °Cd) and Sampling 2 (512 °Cd) but root mass in the top layers increased from 0.2 g at Sampling 1 up to almost 1.5 g at Sampling 3 (768 °Cd).

When drought was applied in the upper compartment (Fig. 2C, D) nitrate capture was impaired at Sampling 1 (288 °Cd): whereas about 70 % of all nitrate available in a layer could be captured in the control treatment (Fig. 2A), in the drought treatment, only 40 % or less was captured by approximately the same root mass. At Sampling 2, while 100 % of the available nitrate was captured in the top layers in the control treatment, in the drought treatment this percentage was only approximately 60 %. At the last sampling (Sampling 3, 768 °Cd), although root mass was increased significantly in the dry compartment compared to the control, only up to 80 % of the nitrate present in the layer was captured by the roots. The same results were obtained when drought stress was applied in the lower compartment in combination to nitrogen stress in the upper compartment (Fig. 2J).

Model design

Based on the results obtained from the pot trial and analysed above and in Kerbiriou et al. (2013a), a model concept was developed, shown in Fig. 3. This model was built on the assumption that water or

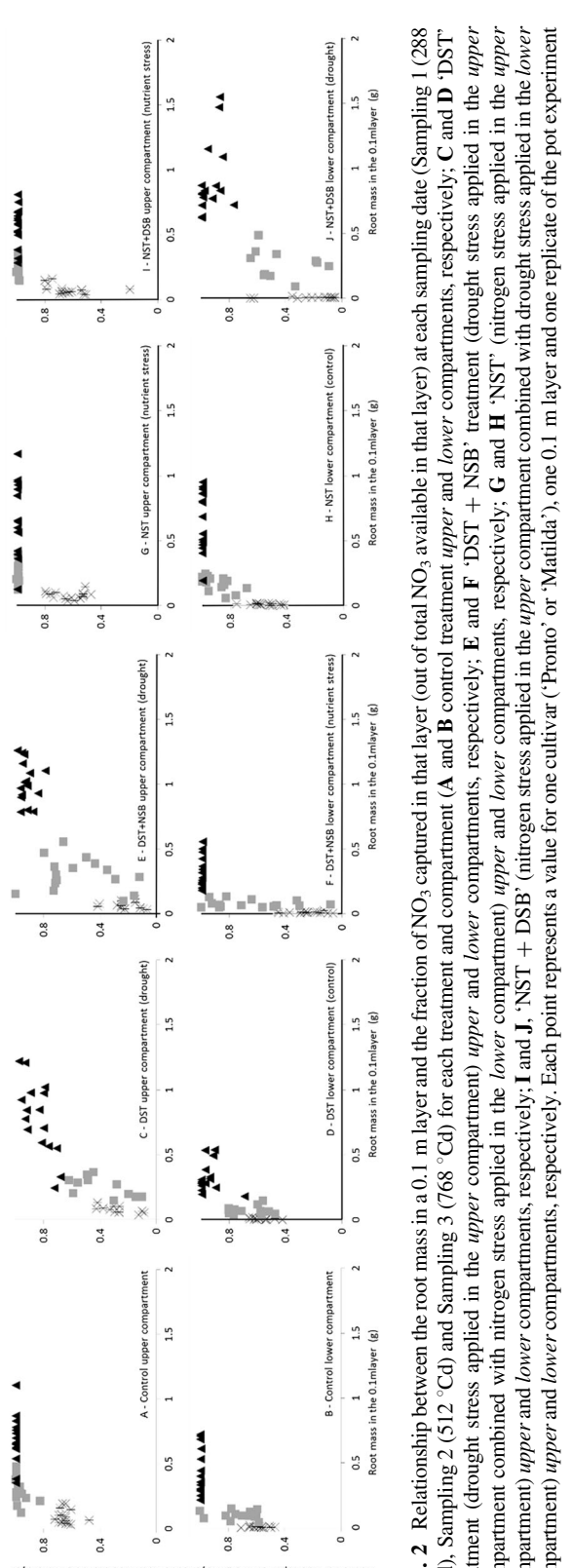

Fig. 2 Relationship between the root mass in a 0.1 m layer and the fraction of NO_3 captured in that layer (out of total NO_3 available in that layer) at each sampling date (Sampling 1 (288 °Cd), Sampling 2 (512 °Cd) and Sampling 3 (768 °Cd) for each treatment and compartment (**A** and **B** control treatment *upper* and *lower* compartments, respectively; **C** and **D** 'DST' treatment (drought stress applied in the *upper* compartment) *upper* and *lower* compartments, respectively; **E** and **F** 'DST + NSB' treatment (drought stress applied in the *upper* compartment combined with nitrogen stress applied in the *lower* compartment) *upper* and *lower* compartments, respectively; **G** and **H** 'NST' (nitrogen stress applied in the *upper* compartment) *upper* and *lower* compartments, respectively; **I** and **J**, 'NST + DSB' (nitrogen stress applied in the *upper* compartment combined with drought stress applied in the *lower* compartment) *upper* and *lower* compartments, respectively. Each point represents a value for one cultivar ('Pronto' or 'Matilda'), one 0.1 m layer and one replicate of the pot experiment

nitrogen shortage in the soil leads to different root responses and that temporal and spatial dimensions influence the physiological mechanisms regulating resource capture and use efficiency.

External conditions as well as the internal status of the plant determine the partitioning of assimilates between the shoot and the root

In the model concept, the pool of assimilates produced by photosynthesis is influenced by environmental conditions and the ratio between the actual and potential plant transpiration. Besides, the nutritional status of the plant, measured as shoot [N] also influences the partitioning of assimilates as young plants vs. mature plants do not have the same nutritional requirements; more developed plants may require higher levels of nitrogen to maintain their growth rate and would therefore invest more assimilates into root growth to sustain their needs.

Spatial root growth throughout the soil profile is influenced by the local soil nitrate concentration

The portion of assimilates allocated to total root growth indirectly determines root proliferation into different soil layers. A fixed fraction of them are allocated to vertical soil exploration. In each soil layer, the nitrate concentration is determined by the amount of nitrate present in the layer and the moisture content of that layer. Nitrate concentration in a soil layer varies over time due to nitrate and water capture by the plant, and potential leaching to a lower soil layer. The partitioning of the total root mass in different soil layers depends on the nitrate concentration in the layer; as observed previously in the pot trial, root growth may occur in an N-rich layer (as opposed to an N-poor layer) when the plant requires nitrogen capture to sustain its growth rate; based on the same amount of nitrate present in two layers, root growth may increase in the driest layer as its nitrate concentration increases when its moisture content decreases.

Water capture mechanisms are purely physical and only partially impact nitrate capture processes

Environmental conditions influence transpiration which determines the overall water capture from the soil; combined with the moisture content within a

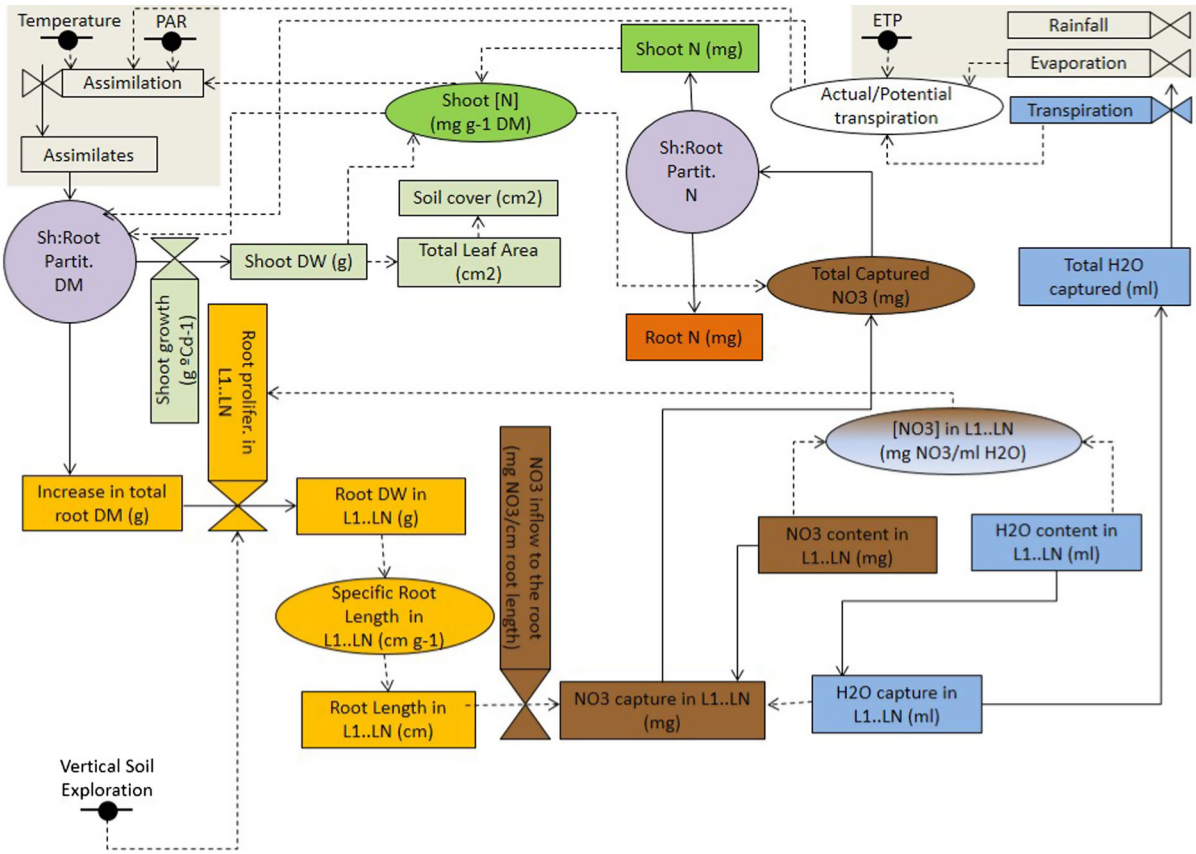

Fig. 3 Model concept flow chart (*Sh* shoot, *Partit*. partitioning, *DM* dry matter, L1… LN: soil layer L1 to soil layer LN); *PAR* photosynthetically active radiation, *N* nitrogen, *ETP* evapotranspiration. Continuous lines indicate a flow of material; dotted lines indicate a flow of information

layer, transpiration indirectly affects the amount of water to be captured in a given soil layer. As shown previously, in optimal conditions additional root growth does not lead to additional nitrate capture, thus in the model concept, nitrate capture in a certain layer is only impacted by the moisture content of that layer and the amount of nitrate available in that layer.

The overall amount of N captured below-ground is a key element by influencing the nutritional status of the plant and determining spatial nitrate capture

Overall nitrate captured in all layers is then allocated to the shoot (impacting shoot [N]) or to the roots. The shoot [N] then regulates the amount of N to be captured below-ground as a feedback loop as the nutritional status of the plants determines the quantity of resources required to sustain the shoot growth rate. The total amount of nitrogen captured below-ground may also affect the amount of nitrogen captured in a specific layer as if the whole requirement is not met by resource capture in certain layers, it may increase the capture in other layers as a compensation mechanism.

Assessing genetic variation in physiological processes determining resource capture and use

Variation in physiological mechanisms involved in resource capture and use efficiency

Table 3 summarizes the results for the above-ground and below-ground measurements performed on the 148 cultivars at intermediate and final harvest during the four field trials.

Under optimal growing conditions (Trial 2, 2010) the highest dry matter production and highest nitrogen

Table 3 Mean, minimum (Min) and maximum (Max) values for soil and plant measurements at intermediate (Inter.) and final (Final) sampling for the four field trials across the population of 148 lettuce cultivars

Sampling	2010				2011			
	Trial 1		Trial 2		Trial 1		Trial 2	
	Inter.	Final	Inter.	Final	Inter.	Final	Inter.	Final
Soil [H_2O] left in layer (v:v)								
0.0–0.1 m	0.16	0.07	0.13	**0.20***	0.15	**0.09**	**0.13**	**0.27**
Min–Max	0.06–0.45	0.00–0.60	0.08–0.32	0.09–0.32	0.12–0.24	0.06–0.14	0.11–0.17	0.20–0.36
0.1–0.2 m	0.20	0.08	0.16	0.18	0.17	**0.09**	**0.16**	0.26
Min–Max	0.06–0.31	0.00–0.29	0.13–0.26	0.10–0.30	0.14–0.26	0.07–0.19	0.12–0.17	0.22–0.30
0.2–0.3 m	0.20	0.08	0.18	0.15	0.18	**0.10**	**0.17**	0.24
Min–Max	0.14–0.41	0.00–0.42	0.11–0.34	0.01–0.41	0.13–0.26	0.07–0.16	0.12–0.19	0.20–0.29
0.3–0.4 m	0.19	0.10	0.18	0.15	0.15	**0.11**	0.14	0.20
Min–Max	0.12–0.32	0.01–0.38	0.12–0.29	0.09–0.32	0.08–0.24	0.08–0.16	0.10–0.17	0.16–0.28
Water left over the 0.4 m soil profile (mL)	740	318	**646**	689	652	**388**	474	**959**
Min–Max	474–1,048	167–784	440–855	464–1,000	637–725	335–628	407–667	694–1,093
Soil [NO_3] left in layer (mg kg^{-1} soil)								
0.0–0.1 m	178	51	132	**129**	163	103	175	117
Min–Max	89–245	6–261	31–393	23–247	71–259	0–530	62–307	4–215
0.1–0.2 m	141	33	160	**115**	110	24	151	**38**
Min–Max	76–278	6–272	11–278	9–228	56–307	0–260	68–217	6–155
0.2–0.3 m	157	49	**164**	**111**	**105**	21	**154**	**50**
Min–Max	13–230	7–143	71–251	9–328	51–164	0–164	71–230	4–196
0.3–0.4 m	136	67	148	120	119	101	**149**	**57**
Min–Max	82–218	7–338	78–241	38–189	30–199	0–261	32–306	3–216
NO_3 left over the 0.4 m soil profile (g)	0.44	0.14	0.44	**0.34**	0.36	0.18	0.42	0.19
Min–Max	0.22–0.57	0.03–0.39	0.25–0.60	0.12–0.54	0.27–0.53	0.04–0.62	019–0.58	0.02–0.42

	2010, Trial 1 at final harvest	2010, Trial 2 at final harvest	2011, Trial 1 at final harvest	2011, Trial 2 at final harvest
Plant fresh weight (g)	294	**483**	**344**	**514**
Min–Max	162–539	201–685	193–467	256–785
Plant dry weight (g)	**28.8**	**46.8**	**22.5**	**20.7**
Min–Max	18.3–51.2	22.0–74.5	16.2–28.2	10.7–42.6
Plant [H_2O] (g H_2O g^{-1} DM)	**9.3**	**9.5**	**14.3**	**24.0**
Min–Max	5.1–16.7	4.3–17.5	9.6–19.8	13.0–33.2
Plant H_2O (g per plant)	265	**436**	**322**	**493**
Min–Max	185–456	186–601	236–430	259–710
Plant [N] (g N kg^{-1} DM)	**24.2**	22.4	**23.8**	**37.0**
Min–Max	7.8–32.2	6.2–36.8	16.7–32.1	28.4–46.8
Plant N (g per plant)	**0.69**	1.03	**0.53**	**0.76**
Min–Max	0.41–1.48	0.29–1.89	0.36–0.80	0.40–1.59
Plant NUE (g DM g^{-1} N in head)	**41.9**	49.4	**42.5**	**27.2**
Min–Max	31.0–56.1	27.2–161.7	31.2–59.9	21.4–35.2

* For values in bold, significant genetic variation was found at $p \leq 0.05$

use efficiency (NUE) at final harvest were achieved. Highest levels of nitrate left in each soil layer and over the whole soil profile at final harvest were also recorded during this trial. Significant genetic variation was found in fresh and dry yields, plant H_2O and plant [H_2O] as well as in the amount of nitrate left in the soil layers and over the whole profile at final harvest. No genetic variation was found in the NUE in this trial.

Under dry conditions (Trial 1, 2010 and Trial 1, 2011), genetic variation was found in all shoot measurements at final harvest, except for fresh yield in Trial 1, 2010. No significant genetic variation was found in soil moisture or nitrate measurements at final harvest for Trial 1, 2010, with relatively mild temperatures, whereas significant genetic variation was found in moisture content in each soil layer and over the whole soil profile at final harvest in Trial 1, 2011 under much colder temperatures.

Under wet conditions (Trial 2, 2011) significant genetic variation was found in shoot measurements at final harvest; such significant genetic variations were also found in the moisture content in the top soil layer (0.0–0.1 m layer) at final harvest, and in soil nitrate measurements in the layers 0.1–0.2, 0.2–0.3 and 0.3–0.4 m at final harvest.

Partitioning the total variance into variance components

The partitioning (%) of the total variance recorded for each trait into different variance components is summarized in Table 4. For the below-ground measurements (soil moisture content in each 0.1 m layer of the 0.4 m soil profile and the volume of water left over the whole 0.4 m soil profile and [NO_3] in each 0.1 m layer of the 0.4 m soil profile and total NO_3 of the whole 0.4 m soil profile) the moment of sampling had the largest contribution to the total variance observed, with 45–89 % of the total observed variance in below-ground traits accounted for by the Y(year) × T(trial) × S(sampling) effect compared to Y × T. This confirms the results in Table 3 showing that differences found in below-ground measurements were much larger between samplings within trials than between trials within a sampling date. For all water measurements, the contribution of the main genotypic effect to the variance was null; the effect of G × Y × T accounted for 1 % of the total variance recorded for both [H_2O] and [NO_3] measured in the 0.2–0.3 m and the 0.3–0.4 m layers. Two percent (2 %) of the total variance recorded in the [NO_3] measured in the 0.0–0.1 m layer was due to genotypic effect only (1 %) or to G × Y (1 %). A small proportion of the total variance within the total amount of NO_3 left over the whole 0.4 m soil profile was attributed to the genotypic effect alone (1 %) or to the G × Y × T interaction (1 %).

The largest proportion of the total variance recorded for shoot measurements was attributed to the effect of the growing conditions within a single environment (i.e., Y × T) with 35–71 % explained by Y × T. The main year effect ('Y') explained 45 % of the variance recorded in shoot dry weight across trials, and 56 and 34 % of the total variance observed in plant [H_2O] and plant H_2O. The main genotypic effect ('G') explained 1 % of the total variance recorded in shoot dry weight and plant N, 2 % of plant [N] and 4 % of shoot fresh weight. The largest proportion of the total variance attributed to the interactions between genotypic effect and single growing environment ('G × Y × T') was found for plant fresh weight (11 %).

Table 4 therefore shows that the effects of sampling time and environmental conditions during growth and their interactions were causing the largest proportions of the total variance of the below-ground traits. For above-ground traits, year and year × trial were important variance components. Nevertheless, within trials there were significant cultivar differences that were relevant for practice (bold data in Table 3) and the ranges of the cultivar means were also large in many cases. Almost all above-ground variables and several below-ground variables showed significant cultivar effects within trials. However, the residual variances were large for all below-ground variables and several of the above-ground variables. Moreover, when cultivar means of the variables of one of the four trials were plotted against cultivar means of the variables in one of the other three trials then the correlations were very small and the rankings were very inconsistent, demonstrating very large genotype × environment interactions (relations not shown). This type of inconsistent genotype × environment interactions were also demonstrated by Des Marais et al. (2013) (their Fig. 1E). Moreover, in-depth analysis of the above-ground and below-ground data on presence of nitrogen showed that combining information on uptake by the plant and residual soil N does not provide full insight into the dynamics of

Table 4 Partitioning of variance components (as % of the total variance) for below- and above-ground variables between Genotype ('G'), Year ('Y': 2010 or 2011), Trial ('T': Trial 1 or 2 within a year), Sampling ('S': Intermediate or Final Sampling within a trial), the interactions between components ('G × Y', 'Y × T', 'G × Y × T', 'Y × T × S' and 'G × Y × T × S'), and the residual error (Res.)

	G	Y	G × Y	Y × T	G × Y × T	Y × T × S	G × Y × T × S	Res.	Total
Soil [H_2O] left in layer (v:v)									
0.0–0.1 m	0.0	0.0	0.0	0.0	0.0	83.9	0.0	16.1	100
0.1–0.2 m	0.0	0.0	0.0	0.0	0.3	89.4	0.0	10.3	100
0.2–0.3 m	0.0	0.0	0.0	0.0	0.7	81.5	0.0	17.8	100
0.3–0.4 m	0.2	0.0	0.1	0.0	1.0	62.5	0.0	36.3	100
Water left over the 0.4 m soil profile (mL)	0.0	0.0	0.0	0.0	0.0	79.9	0.0	20.1	100
Soil [NO_3] left in layer (mg kg^{-1} soil)									
0.0–0.1 m	0.9	0.0	0.6	0.0	0.1	51.1	0.0	47.3	100
0.1–0.2 m	0.4	0.0	0.4	0.0	1.1	76.4	0.0	21.7	100
0.2–0.3 m	0.4	0.0	0.4	0.0	1.2	78.9	0.0	19.2	100
0.3–0.4 m	0.0	0.0	0.0	0.0	0.0	45.0	0.0	55.0	100
NO_3 left over the 0.4 m soil profile (g)	0.6	0.0	0.2	0.0	1.0	73.8	0.0	24.3	100
Plant fresh weight (g)	4.3	0.0	0.0	62.5	10.6	n.a.*	n.a.	22.6	100
Plant dry weight (g)	1.3	45.2	0.1	40.5	2.4	n.a.	n.a.	10.5	100
Plant [H_2O] (g H_2O g^{-1} DM)	0.2	56.5	0.0	36.3	0.8	n.a.	n.a.	6.3	100
Plant H_2O (g per plant)	0.0	34.3	1.9	53.2	0.0	n.a.	n.a.	10.6	100
Plant [N] (g N kg^{-1} DM)	1.5	5.6	0.0	70.6	0.0	n.a.	n.a.	22.2	100
Plant N (g per plant)	1.4	3.2	0.0	55.4	0.1	n.a.	n.a.	39.9	100
Plant NUE (g DM g^{-1} N in head)	0.0	10.3	0.0	34.7	0.0	n.a.	n.a.	55.0	100

* Not applicable: as shoot measurements were only made at final harvest, the sampling term ('S') was removed from the model

nitrogen in the lettuce crop (analysis not shown). Improved phenotyping supported by modelling is needed to reduce the residual variance and to improve the expression and evaluation of cultivar differences.

Implications of phenotyping results for model development

A model specifically targeting breeding for resource capture under limiting environment

The results of the field experiments showed that under optimal growing conditions (Trial 2, 2010) nitrogen use efficiency above-ground does not seem to be a trait of interest for improvement, as no genetic variation was found in plant [N] (in g N kg^{-1} DM), plant N (in g per plant) or plant NUE (in g DM per g N) (Table 3). In contrast, below-ground traits displayed a higher level of genetic variation and higher repeatability values in limiting growing conditions such as in Trial 1, 2011; this suggests that a mild level of drought or nitrogen stress during growth is conducive to the expression of diverse coping strategies and consequently leads to a broader range of variation in such strategies. On the other hand, harsh growing conditions like in Trial 1, 2010 do not seem suitable as a breeding environment as they suppressed potential genetic variation in resource capture and growth responses. Being able to simulate different growing conditions and their effect on the different traits would thus be useful in breeding programmes targeting specifically organic growing conditions where crops are often subjects to mild and temporary shortage of resources during growth.

Using a model approach to cope with Genotype × Environment interactions

The experimental results obtained in the field trials highlighted the strongly inconsistent cultivar effects

across trials (both within and between years) affecting the expression of the various traits. The physiological mechanisms identified in the pot experiment and their function as integrated in the model design could hardly be retrieved in the field trials results. Especially the combination of the influence of the genetic variation and the impact of the growing conditions made the results of the measurements on moisture and nitrate content over the soil profile very complex to analyse and to understand. As shown in Table 4, the contribution of the genotypic effect on the variance in measurements made on below-ground traits was very limited compared to the impact of the growing conditions, highlighting the inconsistent cultivar differences across trials affecting the expression of the traits measured in these trials—which would make them very difficult to breed for. The measurements made for below-ground traits during the field experiments are hardly possible to integrate as such in a breeding programme, partly because of the enormous amount of labour requirement and partly because of such large residual variances and inconsistent cultivar effects. However, such large datasets provide an excellent basis to build and test the model. Moreover, a model accounting for inconsistent cultivar behaviour across environments would be a useful tool in a breeding programme as it would point out which traits are of interest for a given breeding environment.

Greater details in the interactions between soil resource availability, resource capture and root growth and the genetic variation thereof are needed as a step forward building the model

This being said, the traits involved in resource and use efficiency measured in this study nevertheless displayed large and significant genetic variations within trials; once their dynamics over time and space will be better understood and dissected in more stable variables, they will present an interesting potential for breeding purposes. In particular, the pot trial results shed light on the possible effect of localized change in nitrate concentration and/or moisture content on root growth. The results seem to confirm what was observed previously by Drew et al. (1973) who found that N-rich patches increases lateral root growth in barley; these findings also seems to be in line with the conclusions of Chapman et al. (2011), who found that in *Arabidopsis thaliana*, while higher nitrate concentration increases basal root growth, more water supply increases primary root growth. Overall, the different roles of localized nitrate concentration, and moisture content on root growth should therefore be studied in more detail to enable the model to take into account the interactive effects of these two resources on root growth in space and time.

Additionally, the experiments carried out in this study demonstrated that in lettuce, additional root growth does not necessarily lead to a higher amount of resource being captured in a non-limiting environment (Fig. 2). This is in contradiction with the study by King et al. (2003): their model was based on an exponential relationship between root length density within the soil profile and resource capture. This relationship, however, seems more in accordance with the mechanisms triggered when lettuce roots experience a dry environment. It might also apply to a nutrient that is less mobile in moist soil such as phosphorus. More research is needed to understand exactly the relationships between root growth and the amount of resource captured over time and space in lettuce.

Implications of phenotyping for breeding: What to breed for and in which selection environment?

The findings of this study underline the importance of breeding for below-ground traits in a growth-limiting environment. The lower levels of genetic variation and repeatability in the traits involved in resource capture and use efficiency found in the trial carried out under optimal conditions (Trial 2, 2010; Table 3) show that under optimal conditions, below-ground traits are not crucial for shoot performance. As all resources are available for uptake, no changes in the plant morphological or physiological processes are required to maintain its growth rate. In this system, both plastic (highly adaptable to their environment) and non-plastic (inert to changes in their environment) plants can perform. Therefore, if genetic variation in yield is observed, it might purely be caused by head morphological characteristics and the total amount of resource the plant is able to capture in the soil given its morphological features. In an optimal environment, varieties with larger overall biomass above- and below-ground are more likely to display higher yields than a variety with a lower overall biomass.

In a system in which resources are limiting, results highlighted that not only improved morphological features are necessary to capture the resource (e.g., a larger root system leads to an improved nitrate capture) but also implicitly that plasticity, as the manner a plant adapts to its environment in a timely fashion, in the processes involved in resource capture and use efficiency seems crucial. This concept was already mentioned by Hodge (2004). Therefore, breeding for resource capture and use efficiency should be done in a mildly limiting environment to trigger the expression of genetic variation; moreover more efforts should be put into understanding the dynamics of the responses in root growth, resource capture and use efficiency in time. As lettuce is a short cycle crop, new cultivars require a high level of plasticity in adaptation to their environment, especially to adapt to organic and low-input environments.

Concluding remarks

This study highlighted the following points:

- Root growth in a soil profile with localized resource shortage depends on the resource that is in short supply: root growth in relation to localized nitrate concentration and moisture content should be studied in more detail.
- Resource capture may be improved by increased root growth in a limiting environment only; selection for root traits and resource use efficiency only makes sense in such a limiting environment.
- There is considerable genetic variation in resource capture.
- The interaction between processes in the upper rooted soil layer and the lower rooted soil layer under conditions in which resources are not abundant and not equally distributed should be further investigated.
- Incorporating the time dimension is an important step to identify cultivars which are more plastic in root development and are capable of responding quickly to changes in their environment by adapting their physiological mechanisms and morphological and architectural characteristics.

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