REVIEW PAPER

Breeding crops for improved mineral nutrition under climate change conditions

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Abstract

Improvements in understanding how climate change may influence chemical and physical processes in soils, how this may affect nutrient availability, and how plants may respond to changed availability of nutrients will influence crop breeding programmes. The effects of increased atmospheric CO_2 and warmer temperatures, both individually and combined, on soil microbial activity, including mycorrhizas and N-fixing organisms, are evaluated, together with their implications for nutrient availability. Potential changes to plant growth, and the combined effects of soil and plant changes on nutrient uptake, are discussed. The organization of research on the efficient use of macro- and micronutrients by crops under climate change conditions is outlined, including analysis of QTLs for nutrient efficiency. Suggestions for how the information gained can be used in plant breeding programmes are given.

Key words: Micronutrient, nitrogen, nutrient availability, nutrient use efficiency, phosphorus, quantitative trait loci (QTLs).

Introduction

Sinclair (1992), writing in this journal, outlined views on how climate change could affect plant mineral nutrition. Since that time we have gained considerably more knowledge of the control of nutrient acquisition by plants and have refined our ideas on what the effects of changing climate on plant nutrition may be.

However, assumptions have to be made about what impact climate change will have on above-ground and below-ground physical conditions, with further assumptions as to what the responses of environmental systems, crops, and individual plants will be to these changed conditions. Imperfect knowledge of plant physiology then must be applied to predict what sort of crop plants will be required to optimize mineral acquisition, without compromising on acquisition and utilization of light and water, and resistance to disease and pathogens.

However, one prospect is more tangible. Current yields of crops in many countries have been achieved through the energy-intensive production and use of fertilisers, so breeding plants with higher nutrient-use efficiency (NutrUE: biomass produced per unit of nutrient available) will help lower emissions of greenhouse gases in the production of fertilisers.

Another definite prospect that can be counted on is that as climate changes the geographical ranges of plant species will change, and many areas will become suitable for crops that previously were grown only in other locations. For countries with poorly developed fertilization, adaptation to new crops will present problems, although for those countries where use of fertilisers is well developed adequate nutrition of the crops to ensure high yields should present few challenges (Brouder and Volenec, 2008). Evidence for the ability of countries with well developed use of fertilisers (and other agronomic practices) to adapt to new crops can be seen in the adoption in the UK over the past 50 years of forage maize (*Zea mays* L.), a crop previously associated with countries further south

in Europe or in the US. In 1965 there were only 1000 ha of forage maize in the UK (Bunting, 1978), but in 2013 the area grown in England was 171 000 ha (Defra, 2014). Yields of 15.9–18.4 tonnes dry matter ha^{-1} can be achieved on favourable sites (NIAB, 2014), comparable to yields of 10.6–18.3 tonnes dry matter ha^{-1} seen in 15 site-year combinations in experiments in Germany (Herrmann and Taube, 2005).

Adoption of crops in areas previously unsuitable for them will not require breeding programmes, merely the planting of existing genotypes that are suitable for those areas. However, there will be changes in the cycling of nutrients and in rhizosphere chemistry and biology due to climate change that could have an impact on plant nutrition, and these changes should be considered in plant breeding.

Effects of environmental change on availability of nutrients

Increased temperatures, as well as the increased concentrations of atmospheric CO_2 that cause them, will affect soil microbial physiology, thus altering nutrient cycles and the availability of nutrients for crop growth. Microbial processes are likely to occur more rapidly at warmer temperatures, and as microbes break down soil organic matter at faster rates any minerals associated with it will be released into the soil more quickly than at cooler temperatures.

In experiments in which warming is investigated as a sole effect, N mineralization and the rate of nitrification are enhanced (Dieleman et al., 2012; Bai et al., 2013). Although the rate of metabolism of microbes involved in the N cycle may be higher in warmer conditions, increased temperature does not always increase microbial N in the soil, possibly because soil microbial growth is limited by carbon (Bai et al., 2013). There is no certainty that the increased rate of N mineralization with increased temperature seen in some experiments would be a long-term effect that continued after the experimental period (Dieleman et al., 2012). Indeed, although soil microbial respiration is increased by increased temperature in short-term experiments, over long exposure soil microbial biomass, and soil microbial respiration, may actually be decreased (Bradford et al., 2008).

Where enhanced CO_2 concentrations are investigated alone, increased allocation of carbon below-ground arising from increased carbon assimilation gives more fine roots that are subjected to turnover and grazing by soil organisms, so that there is increased immobilization of soil N (Dieleman et al., 2012). Furthermore, supplies of N, and other nutrients, could be influenced by other processes affected by climate change, such as precipitation and evapotranspiration. In long-term free-air CO_2 enrichment (FACE) experiments, higher CO_2 supply has been shown to lower stomatal conductance (Long et al., 2004; Ainsworth and Rogers, 2007; Leakey et al., 2009), indicating that higher atmospheric CO_2 concentrations would lower transpiration (as would lowered precipitation and reduced soil water content arising from warmer conditions) (Brouder and Volenec, 2008; Leakey et al., 2009). This action could reduce the availability of nutrients such as nitrate that

are acquired by crops mostly through mass flow, although diffusion occurs more quickly at warmer temperatures as viscosity is lower and the movement of ions is quicker. It is possible that increased diffusion of ions to roots could compensate for decreased mass flow, so that crop growth would not be affected (Brouder and Volenec, 2008). In a FACE experiment in which double the ambient CO_2 concentration was supplied to semi-arid grassland, there was decreased transpiration (Nelson et al., 2004), yet plant N uptake and growth over 5 years were higher (Dijkstra et al., 2008). In this experiment there seemed to be more mineralization of soil N under the elevated CO_2, possibly because of the increase in soil water that resulted from transpirational loss being lowered, and the resulting increased availability of N that allowed the plants to utilize the increased carbon supply (Dijkstra et al., 2008). This result is in contrast to the progressive decrease in N availability often seen in experiments with enhanced CO_2 alone, although analysis of a limited number of experiments where warming was combined with enhanced CO_2 was inconclusive as to whether such a decline is seen under these combined conditions (Dieleman et al., 2012).

One group of microbes involved in making nutrients available to plants are mycorrhizal fungi, which will be affected by increased atmospheric CO_2 and warmer temperatures. It is the natural state of many crop species to have arbuscular mycorrhizal (AM) associations (Smith et al., 2011; Smith and Smith, 2011), so any effects on mycorrhizal associations are likely to affect nutrient availability. AM fungi aid uptake of phosphate from low-P soil (Bolan, 1991), and they are involved also in uptake of Zn^{2+} and NH_4^+ (Smith and Smith, 2011). Warmer temperatures apparently remove the inhibition of formation of AM associations by the presence of high phosphate concentrations in soil (Graham et al., 1982), but this condition could give rise to associations where the cost to the plant in exported carbon exceeds the benefit of the extra P acquired for the plant by the fungus.

Increased atmospheric CO_2 concentrations increase the growth of AM fungi (Sanders et al., 1998; Alberton et al., 2005; Drigo et al., 2010), as well as growth of the plants infected. Mycorrhizal tomato (Solanum lycopersicum L.) plants had higher biomass than non-mycorrhizal mutants after 22 days under elevated CO_2, despite the biomass of both types of plant being similar with ambient CO_2 supply (Cavagnaro et al., 2007). However, after 72 days of exposure to the elevated CO_2, the mycorrhizal plants had lower shoot biomass than the non-mycorrhizal mutants (although higher than in ambient CO_2), indicating that despite the high availability of CO_2, the costs of donating fixed carbon to the fungus outweighed the benefits to the plants (Cavagnaro et al., 2007). However, mycorrhizal associations also increase resistance of plants to pathogenic microorganisms, and increase resistance to drought (Smith and Smith, 2011), so the carbon costs in this situation could still benefit a crop.

Increased atmospheric CO_2 stimulates plant growth, which in turn gives a higher N demand. This action seems to stimulate AM fungi and saprophytes that mineralize soil organic matter in low-N soils, and the AM fungi transfer the NH_4^+ ions released to the plants (Cheng et al., 2012). In N-fixing

leguminous species, where symbiotic association with rhizobia gives the plants their own source of N, results of several studies show that increased atmospheric CO_2 concentrations give a big increase in N fixation (Idso and Idso, 2001). However, in legumes reliant on fixation in nodules for their N supply, the potential for increased growth with the increased supply of CO_2 still appears to be limited by N availability as carbon assimilation acclimates to enhanced CO_2 with time yet supply of additional N as ammonium nitrate in experiments overcomes this acclimation (Sanz-Sáez *et al.*, 2010).

Colonization by AM fungi can increase the rate of N fixation in leguminous plants (Mortimer *et al.*, 2008), and may help to overcome N limitation under enhanced CO_2 supply. Goicoechea *et al.* (2014) found that in lucerne (*Medicago sativa* L.) exposed to enhanced CO_2 for 2 weeks, there was not only more carbon assimilation than in ambient CO_2 but mycorrhizal plants had significantly more carbon assimilation than non-mycorrhizal plants. However, after 4 weeks there was no effect of their being mycorrhizal. Acclimation of carbon assimilation occurred in the mycorrhizal plants and not in the non-mycorrhizal plants, so if shortage of N usually limits the enhancement of growth under elevated CO_2 this process was not overcome by mycorrhizal association. Nodulation of leguminous plants actually appears to be suppressed during the early stages of mycorrhizal colonization (Mortimer *et al.*, 2008), and with there being two different symbionts both acting as sinks for fixed carbon, and both potentially able to supply N, the interaction is complex.

Another effect of increased temperature is that soil adsorption of anions is likely to be decreased and adsorption of cations increased (Barrow, 1992). This action could affect uptake of anionic phosphate by plants, as it is strongly adsorbed in soil. When phosphate is supplied it is initially adsorbed in a quick, easily reversed reaction of sorption to the surfaces of clay minerals and iron and aluminium oxides (Barrow and Shaw, 1975a, b). The reaction occurs partly by displacement of water molecules and partly by displacement of hydroxyl ions (Bolan, 1991) and is likely to be lowered at warm temperatures (Barrow, 1992). However, there is a subsequent slower reaction, which immobilizes the phosphate, and at high temperatures the rate of conversion of phosphate to this less exchangeable form is faster than at cold temperatures (Barrow and Shaw, 1975b). At warmer temperatures, phosphate becomes unavailable to plants more quickly after addition to soils, and at any given time there is a lower concentration of phosphate in the soil solution per unit of phosphate supplied than at cold temperatures (Barrow and Shaw, 1975a).

It is uncertain if mycorrhizal plants access immobilized phosphate better than non-mycorrhizal plants or whether they are merely better at accessing phosphate in a low-P soil through expansion into a bigger soil volume (Bolan, 1991). However, it generally seems to be accepted that mycorrhizal or non-mycorrhizal plants access the same forms of inorganic P, including reversibly adsorbed phosphate (Smith *et al.*, 2011). It is therefore unclear if AM associations could be more important for making P available to crop plants with increased temperatures.

Effects of environmental change on plant growth and uptake of nutrients

There is a functional equilibrium between shoot and root growth to optimize a plant's acquisition of resources obtained from the atmosphere and resources obtained from the soil (Brouwer, 1962; Davidson, 1969). Root mass × rate of nutrient absorption is proportional to leaf mass × rate of photosynthesis (Davidson, 1969). Alterations to one of these four parameters automatically changes the equilibrium unless another parameter is changed in the counter direction. Root and shoot growth are increased under higher atmospheric CO_2, with root growth being increased more than shoot growth in non-woody species (Rogers *et al.*, 1994; de Graaff *et al.*, 2006), but root growth in particular is increased only if N is not limiting (de Graaff *et al.*, 2006). As current atmospheric CO_2 concentrations are close to the K_m for Rubisco, increased concentrations should give rise to an increased rate of carboxylation in plants (Long *et al.*, 2004). Increased CO_2 concentrations in FACE experiments give rise to increased rates of light-saturated photosynthesis in either C3 or C4 crops (on average about 13% higher in the former, and 10.5% higher in the latter) (Ainsworth and Rogers, 2007). Carbon gain could be up to 46% higher in C3 plants at the atmospheric concentrations of CO_2 expected for the middle of this century (Leakey *et al.*, 2009). Some increases in carbon gain may also occur in C4 crops, through improvements in water-use efficiency, thereby delaying the onset of water deficit stress (Leakey *et al.*, 2009). The higher efficiency of light-saturated carbon assimilation in FACE experiments gives rise to enhanced plant growth largely through more rapid leaf development, when N is not limiting (Long *et al.*, 2004). Nitrogen is used more efficiently due to the increased C assimilation, but under N limitation the positive effect on growth acclimates over time (Ainsworth and Rogers, 2007; Leakey *et al.*, 2009).

In crops, increased atmospheric CO_2 gives rise to more and longer roots and a higher root:shoot biomass ratio (Rogers *et al.*, 1994; Nie *et al.*, 2013). Shoot and root growth in wheat (*Triticum aestivum* L.) are increased by enhanced CO_2, and total root length is greater (Benlloch-Gonzalez *et al.*, 2014). In contrast, warming alone gives higher shoot mass, but not higher root mass, probably because increased soil microbial activity makes nutrients more available and alters the functional equilibrium of the shoot:root balance (Dieleman *et al.*, 2012). When enhanced CO_2 is supplied at higher temperature, there is still increased root biomass, but not to the same extent as with enhanced CO_2 alone (Dieleman *et al.*, 2012; Benlloch-Gonzalez *et al.*, 2014). Specific P uptake (unit of P taken up per unit root mass) was significantly increased in soil-grown wheat by enhanced CO_2 supply, but it was lowered by increased temperature and by enhanced CO_2 and increased temperature together, as was grain yield (Manoj-Kumar *et al.*, 2012).

If crops maintain stoichiometry between the elements they take up, enhanced biomass production will only occur if growth is not constrained by nutrient supply. However, stoichiometry is not maintained, and plants grown in enhanced

CO_2 contain lower N concentrations (Taub and Wang, 2008). This could be due to the dilution effect arising from increased production of carbohydrates, and decreased N concentrations in leaves of plants grown in enhanced CO_2 seem to be largely due to increased accumulation of non-structural carbohydrates, although a decrease in amounts of Calvin Cycle enzymes may also play a role (Idso and Idso, 2001; Long et al., 2004). They could also be caused by less movement of soluble nitrate to plant roots in bulk flow of water across the soil (Taub and Wang, 2008; McGrath and Lobell, 2013), or to changes to root architecture affecting N uptake (Taub and Wang, 2008). Together with the greater immobilization of soil N under enhanced CO_2 supply, this will give rise to a progressive N limitation in ecosystems with a closed N cycle, but not in agriculture (Dieleman et al., 2012).

In winter wheat grown at 10/15°C, high/low N supply, and CO_2 at 360/700 ppm, nutrient uptake was stimulated by N supply and higher soil temperature, and was depressed by higher CO_2 concentration (Gavito et al., 2001). These actions seem to show that nutrient uptake was affected by plant growth, which was influenced mainly by N supply, and less N was taken up at high CO_2. However, different experiments have given different results regarding N concentrations in crops exposed to enhanced CO_2, possibly due to there being different levels of N supply (Idso and Idso, 2001). Although N and protein concentrations are decreased in leaves and seeds with increased atmospheric CO_2 at low rates of N supply, when plants are supplied with adequate N this dilution effect is not observed (Idso and Idso, 2001). Furthermore, even at low rates of N supply the depressed internal N concentrations arising from enhanced CO_2 appear to be overcome if the experiments are run for long enough, presumably due to adaptation of the root system to the increased CO_2 supply (Idso and Idso, 2001).

Concentrations of other nutrients are also lowered by enhanced CO_2. Concentrations of Ca in the edible parts of food crops are lowered more than N, whereas concentrations of Mg, P, and K are depressed, but less so than N (Taub and Wang, 2008). Other studies have shown that plant concentrations of Mg and S are depressed by high CO_2 more than N, whereas concentrations of Ca, K, and P are depressed but to a lesser extent than N concentration (McGrath and Lobell, 2013). Growing C3 crops at the levels of atmospheric CO_2 predicted for the middle of this century gives lower concentrations of Zn and Fe (Myers et al., 2014). Concentrations of Co, Fe, Mn, Ni, and Zn are depressed by growing in enhanced CO_2, and concentrations of Cu and Si also are depressed in some studies (McGrath and Lobell, 2013). Nutrients for which mass flow across the soil is important for uptake have their concentrations in crop plants depressed by enhanced CO_2 more than nutrients for which mass flow is less important (McGrath and Lobell, 2013).

Many experiments have been carried out on the effects of increased atmospheric CO_2 on mineral accumulation, but fewer have studied the combined effects of increased CO_2 and increased temperature. A study on wheat grown at simulated future CO_2 concentration and temperature predicted for the Indo-Gangetic plains in India by the early second half of this century showed that there could be a possible 9–24% decline in protein, Zn, and Fe concentrations in the grains, as well as a 17% reduction in yield (Manoj-Kumar and Patra, 2010). As the decrease in Fe and Zn concentrations in grains seems to be larger than the decrease in P concentrations, this depression could lower the bioavailability of the Fe and Zn in the human diet, as much of the P in grains is in phytic acid, which immobilizes micronutrients in the gut (Manoj-Kumar, 2011).

With Zn and Fe deficiency being a problem in human nutrition, any reduction in their bioavailability or overall concentration would be undesirable. However, as with N, it is possible that in studies where there were decreases in mineral concentrations in crops grown in enhanced CO_2 this decline may have been due to insufficient supply of the elements in the soil, and therefore their supply in fertilisers may give increased yields of the crops without any decrease in internal mineral concentration (Idso and Idso, 2001). Furthermore, there are genotypic differences within species in the extent of decreases in Zn and Fe with enhanced CO_2 supply, so it should also be possible to breed against this risk (Myers et al., 2014).

Breeding nutrient-efficient crops

Approaches in plant breeding

The major crops have been bred to give optimum yields under the conditions currently prevailing in the main areas of production. Cooper et al. (2014) discuss how breeding of maize has taken place to optimize production in the relatively constant environment of the US Corn Belt, with genetic changes enabling the plants to grow well under current environmental conditions. Firstly, breeding enabled plants to utilize the extra N supplied in fertilisers, but then there was the potential for them to become short of water, so irrigation was practised. There is a genotype × environment (G × E) interaction, but environment is continually changing (in this example through management of N and water supplies) and is accounted for in breeding programmes (Cooper et al., 2014). Traditionally plant breeding has the goal of producing genotypes that are productive across a range of environments, but a significant G × E interaction arising from variations in environment hinders this development (Aspinwall et al., 2014). With climate change, directional selection for adaptation to changes in environment will be required.

Some traits, such as tolerance of crops to herbicides, can be investigated at the biochemical and physiological levels, and the findings can then be scaled up to breed genotypes that express the trait in the field (Sadras and Richards, 2014). Other traits, such as resistance of plants to stresses including water deficit and shortage of N, cannot be scaled up to the field level so easily. If plants are screened for their growth with no stresses imposed, Genotype 1 may outperform Genotype 2, but with a high level of a stress Genotype 2 may outperform Genotype 1. The stress-tolerant genotype pays for its adaptation by not being able to achieve such a high yield when the stress is absent, or is only at a low level. Studies carried out under controlled conditions cannot be used to give a reliable indication of plant performance in the field as the level of

the stress imposed is not controlled, and there will also be additional stresses in the field (Sadras and Richards, 2014). However, in crop species there is some stability in the genotype component of the G × E interaction, and modern wheat cultivars that grow well at high N availability also typically outperform other cultivars at lower N supply (Barraclough *et al.*, 2010; Gaju *et al.*, 2011).

A simple physiological trait like herbicide resistance may be relatively constant between environments. Some morphological traits may also be constant, whilst others show considerable plasticity and vary between different environments. In small grain cereals (wheat, barley, rice, and triticale) plasticity of the components that contribute to grain yield is in the following order: tiller number > inflorescence number = grains per inflorescence > seed size (Sadras and Slafer, 2012). Heritability of grain yield is close to heritability of tiller number (and hence of inflorescence number) and grains per inflorescence, the components of grain number per unit land area. It is a plastic characteristic, whereas variations in seed size are much less plastic. This plasticity means that different experiments can give differences in important traits identified for a species because of the different environments between experiments (Prado *et al.*, 2014). Experimentation identifies which traits are plastic and can be bred into crops to give adaptation to environmental change.

Selecting characteristics for breeding nutrient-efficient crops

Two approaches for selecting characteristics to breed into crops are the analysis of quantitative trait loci (QTLs) in closely related individuals or genome-wide association studies (GWAS) (Mitchell-Olds, 2010). Both are statistical techniques that use linear regression or correlation to match genetic information to phenotype. QTLs are areas of DNA that contain genes that give rise to the trait under consideration or alleles that are linked. If two parent plants that differ in respect of the trait are crossed, and the F2 generation is analysed for markers that distinguish the two parent lines [such as single nucleotide polymorphisms (SNPs)], a marker that is genetically linked to the phenotypic trait segregates with the trait values in a statistically significant manner (Miles and Wayne, 2008). If QTLs for traits associated with plant nutrition are found, a database search for similar DNA sequences that have been identified previously can be performed to find out what genes are involved. These genes then can be manipulated in breeding programmes using marker-assisted selection, where the SNP is selected for. In GWAS, unrelated populations of a species have markers such as SNPs analysed, so that QTLs are identified across the whole genome. This approach works well in model species, where the genome has been mapped. To date, QTL analysis of closely related individuals has been more commonly used than GWAS in agricultural studies, whereas GWAS has been extensively used in the study of human diseases (e.g. The Wellcome Trust Case Control Consortium, 2007).

Development of recombinant inbred lines (RILs) of crop species derived from two or more inbred parents has given the genetic material for QTL analysis. The parents do not need to vary much in respect to the phenotype, but recombination gives rise to expression of different alleles in different individuals. This approach does not have the potential to highlight as many genes as GWAS, but GWAS is not feasible in non-model species that have not been genotyped, and is also difficult in polyploid species (Koprivova *et al.*, 2014). However, the development of associative transcriptomics, where mRNA sequences are studied for identification of polymorphic molecular markers, is now being used in polyploids (Harper *et al.*, 2010; Koprivova *et al.*, 2014).

Studies on QTLs for plant nutritional characteristics under climate change conditions are difficult because of the need to provide enhanced CO_2 concentrations and warmer temperatures in the field. Because of the hardware required they are largely confined to FACE sites or experiments with field-based enclosures. However, studies on previously identified traits, and on the genes co-locating with QTLs, can be carried out in smaller controlled environments. It is also easier to investigate more precise QTLs, so rather than investigating QTLs for the efficiency with which crops use N, for example, QTLs for total N accumulation in shoots, N remobilization into seeds after anthesis, or different enzymes associated with N metabolism can be studied (Chardon *et al.*, 2012). Key proteins such as transporters can be identified without QTL analysis, through analyzing the growth of mutants under changed environmental conditions and investigating the effects of over- or under-expression of the genes concerned on plant nutrition.

Availability of nutrients is an environmental variable that can give plastic responses in plants. Although modern crop species are adapted for growth in soils with high nutrient availability, and the genotype component of the G × E interaction is relatively stable, each cultivar has a different level of efficiency of acquiring and using nutrients. Experiments to identify genes for nutrient efficiency can be carried out at low or high nutrient supply, with genes expressed in either condition being fundamental for nutrient efficiency, and those expressed only at high or low supply being important under that condition. An example of a study analyzing QTLs in relation to nutrition is an analysis of 131 RILs and two parent cultivars of wheat for 20 traits ranging from the agronomic (such as thousand-grain weight), to physiological traits related to K nutrition (Kong *et al.*, 2013). 80% of the 87 additive QTLs in seedlings in hydroponic culture, 92% of the 51 additive QTLs in a pot experiment, and 87% of the 29 additive QTLs in a field experiment were identified only once in the different K treatments imposed (Kong *et al.*, 2013). The QTLs that occurred more than once (15 were identified in most K treatments) can be used for marker-assisted selection of K efficiency (Kong *et al.*, 2013).

Characteristics required for breeding nutrient-efficient crops

The biggest increases in crop yields are likely to come through improvement of primary physiological processes such as carbon assimilation. For example, engineering cyanobacterial

Rubisco into crop plants increases the efficiency of photosynthesis as it has a higher rate of carbon fixation per unit of enzyme than Rubisco from C3 plants at high atmospheric CO_2 concentration (Lin *et al.*, 2014; Price and Howitt, 2014), and offers the prospect of bigger increases in crop yield than would be achieved by breeding for improved mineral nutrition. However, any increase in carbon assimilation would cause a larger demand for N, P, K, and other nutrients, so the efficiency of nutrient use by crops has to be maintained as a minimum requirement to enable increased yields to be achieved.

Nutrient uptake will not only have to be maintained at the current rate following climate change, but due to the dilution effect of the extra carbon assimilation it will have to be enhanced in order to maintain concentrations of nutrients in crop products. Furthermore, due to the energy expended in manufacturing fertilisers it is desirable to breed crops so that the same yields can be achieved with less fertilization as part of a mitigation strategy to lower the emission of greenhouse gases. What is required is crops with high NutrUE.

There is a problem here in that the definitions associated with NutrUE are applied indiscriminately in the literature, although they have been discussed clearly in relation to the efficiency of use of N by plants by Good *et al.* (2004) and Brauer and Shelp (2010), and for use of P by Bovill *et al.* (2013). NutrUE can be defined as the amount of biomass produced per unit of nutrient supplied, and a nutrient-efficient crop is one that produces a large biomass per unit of fertiliser applied. Alternatively, the amount of biomass produced per unit of nutrient in the soil can be measured. The first measure is more common in agronomic research, and gives information on the efficiency of the genotype(s) and of fertiliser use. The second measure is more common in ecological research. NutrUE in agriculture is usually defined as amount of crop product (e.g. grain in a cereal crop) produced per unit of nutrient applied.

If QTLs for NutrUE are analysed, genes identified as being involved can be seen to operate in two different systems. These systems involve either the efficient growth of the plant per unit of nutrient taken up or efficient acquisition of the nutrient from the growth medium relative to the amount present there. NutrUE for seed crops has been described as being comprised of nutrient-acquisition efficiency or nutrient-uptake efficiency (NutrAE or NutrUpE, respectively: amount of total nutrient in the above-ground organs at maturity per unit of nutrient supplied in fertiliser in some studies, or per unit of nutrient available in total in the soil over the growth period in other studies,) and nutrient-utilization efficiency (NutrUtE; seed yield per unit of nutrient taken up) (Moll *et al.*, 1982; Barraclough *et al.*, 2010; Gaju *et al.*, 2011; Hawkesford, 2012; Manschadi *et al.*, 2014; Mendes *et al.*, 2014). An alternative approach is to describe NutrUpE as the difference in nutrient concentration in plants grown with different rates of nutrient supply per unit difference in the rate of supply in the different treatments, and NutrUtE as the difference in yield between the treatments per unit difference in nutrient content of the plants in the different treatments (Hammond *et al.*, 2009).

Analysis of P-use efficiency (PUE) in tropical maize in low-P soil investigated P-utilization efficiency (PUtE) and P-acquisition efficiency (PAE), and showed a strong overlap between QTLs for PAE and PUE (Mendes *et al.*, 2014). This result indicates that PUE in tropical maize with low P supply is determined mainly by the acquisition of P. QTLs for PUE and PAE were located near candidate genes for root development (Mendes *et al.*, 2014). In a study of a large number of genotypes of *Brassica oleracea* L. there were significant positive correlations between lateral root length and measures of efficiency of P uptake (Hammond *et al.*, 2009). Plants can be efficient in P acquisition through their root architecture, being mycorrhizal, having efficient uptake systems, or manipulating the rhizosphere to make phosphate more available from otherwise inaccessible forms. This manipulation can include exudation of organic acids and phosphatases (Mengel and Kirkby, 2001).

Effects of root architecture and manipulation of the rhizosphere may be more important for increasing efficiency of uptake of immobile ions like phosphates than uptake of more mobile ions like K. In the study by Kong *et al.* (2013), wheat was grown in hydroponic, pot, and field experiments. If plants are grown in hydroponics or in soil to study a nutrient that is not readily accessible from soil, such as P or Fe, identifying QTLs for growth and NutrUE that occur in low-nutrient treatments in soil but not in hydroponic culture would highlight genes that are involved in mechanisms of making the nutrient available from the soil.

Improvements in PUE may be obtained by breeding crops with roots that proliferate in the top soil, whereas N-use efficiency (NUE) would be improved by roots that go down steeply to deeper positions in the soil and that are metabolically cheap to produce. However, K-use efficiency could be improved by breeding for root systems intermediate between these two types (White *et al.*, 2013). If there is decreased transpiration with increased CO_2 supply, soil water could increase at depth, so selection of deep-rooted species may be advantageous anyway (Nelson *et al.*, 2004). If increased temperatures favour diffusion of nutrients in soil over mass flow, root systems with higher root length density, higher root hair density, and longer root hairs could be advantageous (Zhang *et al.*, 2007). If higher temperatures increase adsorption of phosphate in soils, root systems with enhanced capacity to desorb phosphate are required. These characteristics will be required in root systems that are likely to have higher growth under increased CO_2 supply and increased temperature (Fig. 1).

As roots and shoots are in a functional equilibrium with each other, differences in shoot activity between genotypes as well as differences in root system activity could affect nutrient acquisition. A QTL for shoot dry mass co-locates with measures of PUE in *B. oleracea* (Hammond *et al.*, 2009), and in wheat shoot growth is a key component of NUE under low N supply (Kamiji *et al.*, 2014). The effect of shoot growth can be seen in the difference in growth between winter and spring wheat cultivars. In bread wheat, a QTL for NUE co-locates with two alleles of the *Vernalization1* (*VRN1*) gene, *Vrn-A1* and *Vrn-D1*, that are involved in vernalization (Quraishi *et al.*,

2011). QTLs for other yield traits under contrasting conditions of high and low N supply co-locate with *Ppd-B1* and *Ppd-D1* alleles of the *Photoperiod1* (*PPD1*) gene involved in the control of flowering by daylength (Laperche *et al.*, 2007). Wheat plants in which *Vrn-A1* and *Vrn-D1* are expressed do not have the requirement for a cold period in order for flowering to occur. Expression of the *Ppd-D1a* allele gives earlier flowering, so enabling the life cycle of the plant to be completed more quickly (Cockram *et al.*, 2007; Distelfeld *et al.*, 2009; Gaju *et al.*, 2011). Although these alleles co-locate to QTLs for NUE, their main effect is on grain yield, which is generally lower in spring wheats than in winter wheats. Early-flowering genotypes with increased *PPD1* expression may grow better in Mediterranean environments, where shortage of water limits growth in the summer, but they do not yield as well as the later-flowering genotypes in northern Europe

where rainfall during the summer is higher (Cockram *et al.*, 2007; Gaju *et al.*, 2011). However, if climate change brings lower summer rainfall to northern Europe the early-flowering genotypes would perform better here also, although later frosts in spring would make genotypes with increased *VRN1* expression less suitable.

Modern 'stay-green' cultivars of grain crops, in which photosynthetic leaf area is maintained for longer and which form more grains, have higher grain yield than traditional cultivars (Borrell *et al.*, 2001). In maize, plasticity in grain yield is in the following order: kernel-desiccation rate = grain-filling period > moisture concentration at physiological maturity > kernel biomass accumulation > kernel weight > maximum kernel water content (Prado *et al.*, 2014). As the grain-filling period is important in maize, it is not surprising that new stay-green cultivars have higher grain yield than traditional cultivars. If
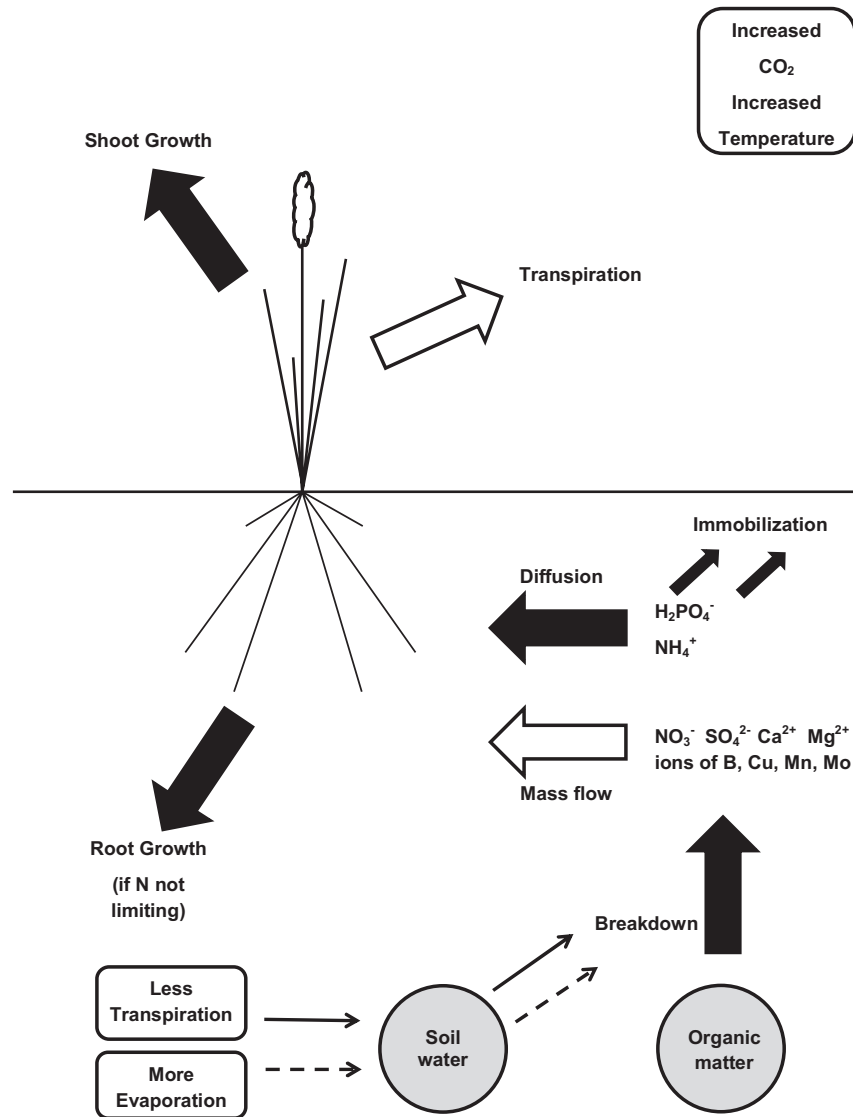

Fig. 1. Predicted effects of climate change on nutrient availability and plant growth. Variables affected by climate change are shown in boxes, and soil pools in circles. Processes positively affected by climate change are shown with solid block arrows, and processes negatively affected by climate change with open block arrows. Positive effects on processes are shown with black line arrows, and negative effects with dotted line arrows. Processes and pools are not to scale. Decreases or increases in rainfall with climate change (dependent on geographic location) will interact with the effects of transpiration and evaporation on soil water pools. Mycorrhizal infection may increase with warmer temperatures, and lower effects of N and P limitation in nutrient-poor soils.

plants were to continue photosynthesizing for longer without taking up any more N, N utilization efficiency (NUtE: grain yield per unit N taken up by the plant) would be higher, giving higher NUE but lower N concentration in the grains. Over the past few decades increases in NUE of cereal crops have come about at the expense of reductions in grain N concentration (Barraclough *et al.*, 2010; Ciampitti and Vyn, 2012; Ning *et al.*, 2013; Manschadi *et al.*, 2014). There are negative correlations between shoot yield and shoot P concentration in *B. oleracea* (Hammond *et al.*, 2009).

However, as stay-green cultivars have higher sink strength through forming more grains than traditional cultivars, there is the potential for more nutrients to be acquired from the soil as well as more carbon assimilation occurring. In 27 winter wheat cultivars grown at seven field sites in northern France, 84% of the N in harvested grain arose by mobilization from leaves and stems and 16% from soil uptake after anthesis (Bogard *et al.*, 2010). There was variation between cultivars, and in a study of wheat cultivars in Kentucky the proportion of N in the spike at harvest arising from mobilization varied between 51 and 91% (Van Sanford and MacKown, 1987). In an analysis of data from field experiments worldwide, Ciampitti and Vyn (2012) showed that in 'old era' (experiments carried out between 1940 and 1990) maize cultivars, 50.7% of biomass was already formed by silking, and 66.2% of final N content was already present at that time; and in 'new era' (experiments between 1991 and 2011) cultivars, 48.4% of biomass was formed by silking time, and 63.2% of the final N content was already present. A large proportion of N in maize grains must therefore arise from remobilization, although the standard deviations in the proportions of N in the plants at silking (16.9, *n* = 245, old era plants; 11.5, *n* = 307, new era plants) indicate that there is potential for manipulation of post-silking N uptake by breeding. There is more uptake of N, P, and K (Borrell *et al.*, 2001; Ning *et al.*, 2013) in stay-green maize after silking than in traditional cultivars, but these nutrients function to enable photosynthesis to continue rather than being partitioned immediately into grains (Ning *et al.*, 2013). Modern winter oilseed rape (*Brassica napus* L.) hybrids have a superior NUE to earlier cultivars; they continue to take up N for a longer growing period, which enables growth to continue for longer without N-limitation occurring, and they also have good translocation of N from vegetative to reproductive organs (Koeslin-Findeklee *et al.*, 2014).

Breeding cereal crops for increased yields has already given grains with lower concentrations of N and other nutrients, and increased atmospheric CO_2 concentrations are likely to exacerbate this effect. Enhanced CO_2 in FACE experiments gives more carbon assimilation per unit of leaf N in C3 plants, because their Rubisco has a higher substrate concentration, and also there is less loss of CO_2 through photorespiration (Leakey *et al.*, 2009). C3 crops grown in higher CO_2 concentrations will have increased NutrUE and lower concentrations of protein and micronutrients than plants grown in low CO_2 concentrations (Taub *et al.*, 2008; Manoj-Kumar and Patra, 2010; McGrath and Lobell, 2013; Myers *et al.*, 2014).

This result will be deleterious for human nutrition, although in bioenergy crops grown primarily for carbohydrates or fatty acids it would advantageous as more of those compounds would be produced per unit of nutrient supplied (Chardon *et al.*, 2012). Food crop species could be selected for good remobilization of nutrients to the seeds under climate change conditions, and this result could be achieved by selecting for high nutrient harvest index (the amount of nutrient in the harvested product per amount of the nutrient in the total plant biomass) (Ciampitti and Vyn, 2012; Hawkesford, 2012). In low N supply, spring barley (*Hordeum vulgare* L.) genotypes that have high NUE do so because they have high NUtE, although at higher rates of N supply either NUtE or NUpE (N-uptake efficiency), or both, are important (Beatty *et al.*, 2010). Differences in NUE between maize genotypes were due to NUpE at high rates of N supply, and NUtE at low N supply (Moll *et al.*, 1982). However, the cultivar differences in mobilization of N in the experiments of Van Sanford and MacKown (1987) did not contribute to significant differences in final spike N concentration, which arose from differences in N uptake.

The high proportion of N in wheat grain already in the plants at anthesis prompted Bogard *et al.* (2010) to suggest that increasing N acquisition through root activity after anthesis would be the best way to improve grain protein content. Given the negative relationship between grain yield per shoot N content and grain N concentration in wheat, it will be difficult to avoid lowering grain yields in trying to increase or maintain grain protein concentration, so fertiliser N application will have to remain high (Barraclough *et al.*, 2010). Nitrogen uptake efficiency decreased in spring barley with an increase in N supply (Beatty *et al.*, 2010); hence, the efficiency of N uptake at higher rates of supply should be improved. To avoid the dilution effect of increased growth from increased carbon assimilation, an increased specific uptake rate [contrary to the decrease seen by Manoj-Kumar *et al.* (2012)] should be selected for, although if root growth is increased under climate change conditions, merely maintaining specific uptake rate may have an equivalent effect. There are already breeding programmes for increased micronutrient concentrations in crop products, such as the HarvestPlus programme that aims to increase concentrations of Fe, Zn, and provitamin A in staple food crops (Hirschi, 2009; Rawat *et al.*, 2013), and this development needs to be further considered for high CO_2 conditions.

Genes that may be manipulated for the efficient use of P are noted by Bovill *et al.* (2013), and for N by Brauer and Shelp (2010). Rothstein *et al.* (2014) list 22 gene constructs that could potentially improve the use of N by plants that have already been investigated. A long-term aim of plant breeding has been to enable cereal crops to carry out N fixation in the same manner as leguminous plants to lower the requirement for N fertilisers. It could be achieved theoretically either by breeding functional nitrogenase into cereals or by breeding the capacity to form nodules into them (Rogers and Oldroyd, 2014), although the process will be difficult.

Specific targets for plant breeding could include the following:

(i) Improve PAE by increasing the density of roots in the top soil layers, to alleviate the decreased availability of phosphate under climate change conditions. However, good growth of the main root will still be required to enable plants to access water (and the nitrate dissolved in it) at depth. Breeding for responses to strigolactones, which signal nutrient deficiency in plants and promote growth of lateral roots and root hairs, could facilitate root branching and root hair formation (Smith, 2013). Exudation of strigolactones promotes AM infection, so this may also bring about enhanced symbioses that would aid uptake of P, Zn, and some N forms.

(ii) Increase the time of shoot activity, for example with the stay-green habit. However, root activity has to be maintained over the extended growth period, and it is also desirable to increase the specific uptake rate of essential nutrients. Effectiveness of root N uptake after anthesis may be affected by root architecture, regulation by plant N status, and the continuing delivery of photoassimilates to the roots (Bogard *et al.*, 2010), and a precise physiological target for breeding long-lasting N uptake into plants has not yet been determined.

(iii) As increased carbon assimilation with climate change could lead to dilution effects for other elements, enhanced NutrUtE and better remobilization of elements already within plants will be required. Overexpression of NADH glutamate synthase in wheat to improve grain weight or grain protein content through improved N remobilization has been the subject of a European patent since 2012 (EP 2534250 A2). Redistribution of essential micronutrients in plants is strongly dependent on metallothioneins (Schiller *et al.*, 2014), genes for which may also be the target for plant breeding.

Conclusions

With changing climatic conditions the geographic range over which plant species flourish will change, and crops will be introduced into areas where they were not grown previously. However, in any given location there will be constraints and opportunities for the growth of species already present.

Increased temperature will affect nutrient cycles, as will changes in soil water content. In those locations where soil water is decreased the availability of soluble nutrients, such as nitrate, that move to plants by mass flow will be decreased. Decreased transpiration of crops arising from lowered stomatal conductance with higher atmospheric CO_2 will lower this availability even more. In contrast, rates of diffusion of less-mobile ions will increase with warmer temperatures, although not for phosphate, which will be increasingly immobilized. However, increased root growth arising from the enhanced carbon assimilation may compensate for decreased availability of nutrients. Furthermore, increased growth of mycorrhizal fungi under high CO_2 supply will aid the uptake of P and other immobile elements.

Enhancement of crop growth may occur with increased atmospheric CO_2, at least in conditions where N is not limiting. However, increased carbon assimilation will cause a dilution effect that lowers the concentration of N and other elements in crop products. This could lower the contents of proteins and essential micronutrients, with consequent effects for human nutrition, unless fertilisers continue to be used at high rates. High fertiliser use conflicts with the requirement to lower the emissions of greenhouse gases in fertiliser manufacture and application, so improvements in NutrUE will be required.

Improvement in NutrUE may be realized in low-intensity agriculture by increasing the efficiency of uptake of nutrients by breeding for suitable root systems. However, in more intensive agriculture, increased NutrUtE from remobilization of N and micronutrients within the plants will be required. Prolonged root uptake and better remobilization of nutrients are targets for breeding, and there is sufficient plasticity of these characteristics in crop species for increases in efficiency to be achieved.

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