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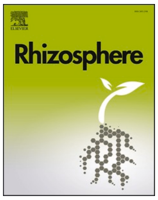

Soil structure has a greater effect on the rooting of wheat (*Triticum aestivum* L.) than nitrogen fertilisation rate or genotype

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ABSTRACT

Despite extensive research over the last century concerning the application of nitrogen fertilizer to support the production of wheat (*Triticum aestivum* L.), our understanding on how this impacts on root growth in subsoils is limited. In this study, we investigated how different rates of nitrogen fertilization (100, 200 and 350 kg/ha N) affected the root and shoot growth of three different wheat genotypes. We collected field soil cores to a depth of 100 cm and scanned them using X-ray Computed Tomography (CT) to quantify the volume of macropores and stony material. The collected soil cores were then destructively segmented to determine root number density. Our results showed nitrogen fertilization rate had a limited effect on root growth and proliferation in both the topsoil and subsoil. Furthermore, wheat genotype did not play a significant role in determining root growth at any depth, with no significant differences between the different genotypes. However, soil macroporosity was positively correlated to root number density, accounting for 48% of the variation. Our results provide evidence that soil management (e.g. cultivation techniques) may be the key to improving subsoil rooting regardless of crop genotype and nitrogen rates applied.

1. Introduction

Nitrogen is an essential component in the manufacture of plant protein and thus strongly controls plant growth and development (Brady and Weil, 2017; Fageria and Baligar, 2005). Considering this importance, crop management practices over the past century have often focused on improving plant access and use efficiency of nitrogen for the improvement of crop yield. For instance, during the green revolution of the 1950s and 1960s, the provision of industrially produced inorganic nitrogen fertilizers partnered with new semi-dwarf plant genotypes that were resistant to lodging provided an ideal catalyst for a substantial increase in yield of most cereal crops (Evenson and Gollin, 2003; Pimentel, 1996). This yield increase in wheat, however, has since plateaued as wheat across many European countries now receive near optimum levels of nitrogen fertilization (Brisson et al., 2010). Further increases in nitrogen application rates not only produce diminished returns on investment in terms of plant productivity, but can also result in environmental issues as the excess nitrogen not taken up by plants may be lost to leaching and contaminate groundwater (Pimentel, 1996). Excess nitrogen fertilizer application can also contaminate surface water bodies leading to eutrophication and damaging fragile freshwater ecosystems (Huang et al., 2017; Khan and Mohammad, 2014). Furthermore, excess nitrogen fertilization also necessitates an increase in the industrial manufacture of nitrogen fertilizer, which uses a large amount of energy. This energy is often derived from fossil fuels thereby increasing global greenhouse gas emissions and consequently exacerbating global warming (Sigurnjak et al., 2017). It is thus prudent to devise strategies that enhance plant nitrogen uptake from soil to improve plant yield, quality, and minimise negative environmental effects.

The development of strategies to maximise the use of available nitrogen in wheat has been the subject of many studies, with several highlighting the importance of improving nitrogen capture by wheat roots (Hawkesford, 2014; Kant et al., 2011; Kaur et al., 2022; Mcallister et al., 2012). Traits such as high root length density, deep rooting,

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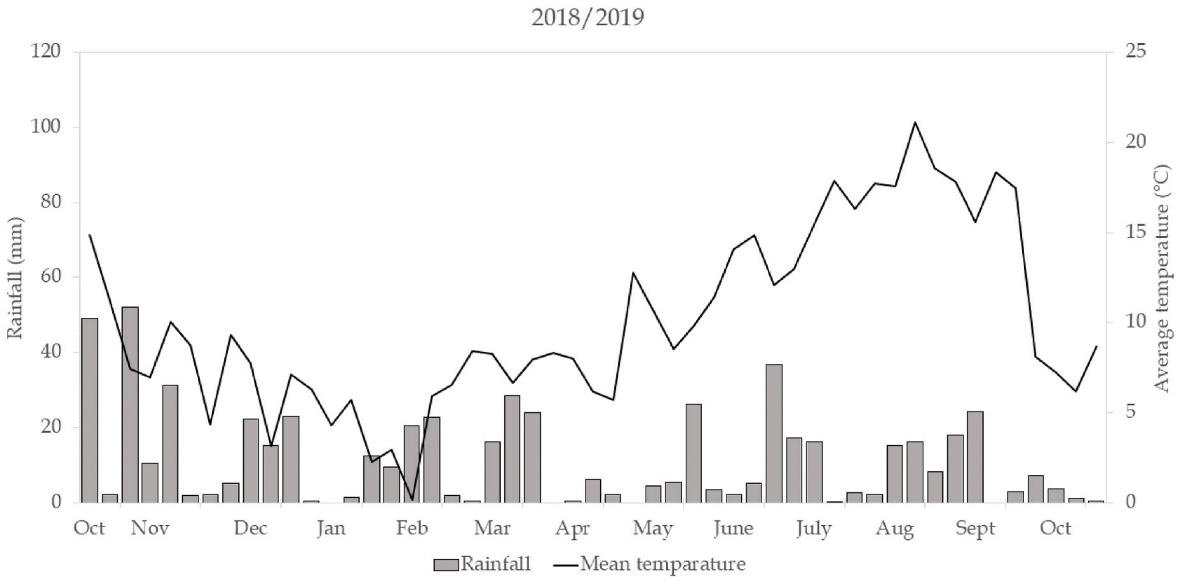

Fig. 1. Average rainfall and temperature for the field sites at Rothamsted Research during the period of growth in this study.

increased root biomass and development of genotypes with roots that have enhanced nitrogen uptake capabilities, have been suggested to achieve this (Kant et al., 2011; Van Der Bom et al., 2020). Of these strategies, deep rooting is one of the most appealing methods as it not only enables subsoil nitrogen recovery, but also allows wheat roots access to other subsoil nutrients and water, thereby conferring improved performance under suboptimal topsoil nutrient and moisture conditions (Guo et al., 2020; Kuhlmann et al., 1989; Li et al., 2022). In this regard, modern semi-dwarf wheat varieties have been shown to exhibit significantly shallower rooting depths and reduced root length density which may reduce their ability to tap into subsoil nitrogen reserves (Austin et al., 1980; Aziz et al., 2017; Fradgley et al., 2020). However, Aziz et al. (2017) observed modern Australian varieties tended to compensate for their smaller root system size by improving nitrogen uptake per root area. However, their shallow rooting may still limit the ability to thrive under suboptimal topsoil conditions.

Many species, including wheat, exhibit significant rooting plasticity that enables them to adapt to variable edaphic conditions (Fromm, 2019; Gruber et al., 2013). Several studies have investigated the response of the roots of various wheat lines to different levels of fertilisation (Comfort et al., 1988; Svoboda and Haberle, 2006; Wang et al., 2014) and although the results are often varying, they generally show root growth responds positively to the application of moderate levels of nitrogen, especially at shallow depths (0–30 cm). Roots tend to show localised proliferation in areas of nitrogen application (often near the topsoil). On the other hand, in the subsoil, root growth is often negatively correlated to increased surface nitrogen application with preferential root growth at the surface limiting plant investment in roots at depth (Comfort et al., 1988; Gregory, 1994; Svoboda and Haberle, 2006). Alternately, in soils with limited availability of nitrogen, wheat roots tend to produce slender, longer roots thus increasing their root length per unit area which increases their nitrogen foraging capacity (Bosemark, 1954; Fageria and Moreira, 2011). In practice, however, neither extremes of nitrogen availability are desirable in wheat production, with an optimal level of nitrogen being ideal to attain both preferential root growth whilst limiting nitrogen pollution (Yang et al., 2017).

Nitrogen does not have an impact on wheat root growth in isolation but rather its effects are dependent on several other edaphic factors such as soil structure, moisture content and temperature (Gregory, 1994; Gregory et al., 2005). Soil structure especially plays a critical role in determining wheat root response to nitrogen application by influencing root access to nutrients (Nawaz et al., 2012). Soil compaction is known to reduce plant response to nitrogen fertilization, often leading to an increase in the total nitrogen fertilizer applied in compacted soils (Douglas and Crawford, 1993; Ishaq et al., 2001; Lipiec and Stepniewski, 1995). The reduced responsiveness of plants to nitrogen is thought to be a result of compaction-induced restriction of root growth coupled with an increase in denitrification as a result of poor aeration (Soane and van Ouwerkerk, 1995). Nitrogen losses in compacted soils are also known to occur due to increased surface water runoff carrying nitrogen with it, and eroding nitrogen-containing soil; the compacted soil also restricts percolation of dissolved nitrogen and thereby limiting plant access to the nutrient. The response of plants to nitrogen is also dependent on moisture availability, with reduced soil moisture limiting plant response to nitrogenous fertilizer (Elazab et al., 2016; Wang et al., 2014).

Soil structure itself has a profound effect on wheat root growth even when soil nutrient status is kept constant. This is because roots have to overcome the mechanical resistance imposed by a soil's structure to elongate and thereby proliferate into it (Jin et al., 2013; Lucas et al., 2019). Soils with a relatively high mechanical resistance such as compacted or very dry soils limit the rate of root growth thus resulting in poor plant performance. The tolerance of roots to high mechanical resistance, however, varies both between and within species, with tap-rooted plants often considered to have roots with higher compaction tolerance as compared to fibrous roots (Colombi and Walter, 2017; Jabro et al., 2021). Plant rooting in the subsoil is mainly affected by the availability of connected macropores as an increase in overburden pressure with depth increases soil mechanical impedance, thus forcing roots to preferentially grow in existing pores (Gao et al., 2016). As a result of this preference, wheat root growth is directly correlated to the porosity of the soil with Zhou et al. (2021) showing that macroporosity plays a more important role in root growth in the subsoil as compared to genotype.

Alongside the importance of soil structure, the stoniness of a soil also effects wheat root growth. Although studies investigating the impact of stones on root growth are relatively scarce, existing evidence suggests stones generally reduce root growth by producing conditions similar to those imposed by high mechanical impedance (Babalola and Lal, 1977; Qin et al., 2015). These effects are more pronounced when stones are coarse and occupy a volume greater than 10–20% of the total soil volume (Poesen and Lavee, 1994), reducing the effective soil volume thus limiting the nutrient and water-holding capacity. For durum wheat (*Triticum durum. L*) roots, stones have been shown to reduce root length

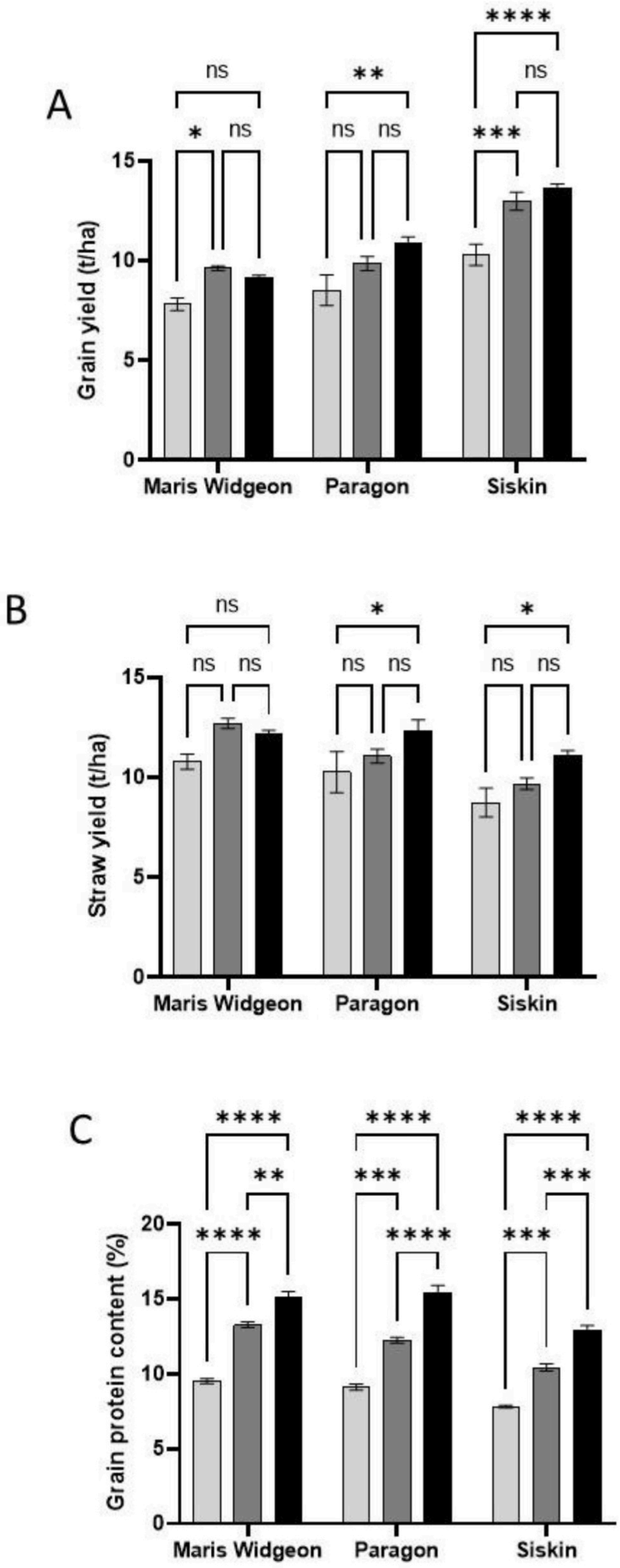

Fig. 2. Grain yield, straw yield, and grain protein content for the three different wheat lines grown with different levels of nitrogen fertilization (100 (light grey), 200 (dark grey) and 350 (black) kg/ha N. Error bars indicate standard error of the mean (SEM). ns: not significant. Symbols indicate significant difference as compared to the wild type; One-Way ANOVA test with post-hoc Bonferroni test, (*=≤0.05, **= ≤0.01, ***=≤0.001, ****=≤0.0001).

and grain yield as a result of the restriction in root zone soil volume (Ercoli et al., 2006). This is however dependent on the mineralogy of stones occupying soil, with stones comprised of chalk and sandstone having a considerable water-holding capacity, and which may also contribute to plant nutrition (Poesen and Lavee, 1994). The effect of stones is also dependent on soil texture as their impact is less severe in finer textured soils such as clay soils compared to sandy soils which have a lower water holding capacity (Babalola and Lal, 1977; Lutz, 1952).

The objective of this study was to investigate how nitrogen rate affects the root growth of three different wheat genotypes in a stony soil. We used X-ray Computed Tomography (CT) to quantify the soil macroporosity and stoniness, and to assess their interaction with nitrogen fertilisation rate and the effect on wheat root growth. We hypothesized wheat root growth would be enhanced by increasing the rate of nitrogen fertilisation and thus facilitate improved root and shoot growth. We also hypothesize that wheat root growth would be enhanced by increased macroporosity, whilst being limited by high quantities of stony material.

2. Methods and materials

2.1. Site and plant material description

The field experiment was conducted on Blackhorse Field at Rothamsted Research, Hertfordshire, UK (51°48′34.56″N, 0°21′22.68″W). The soil is classified as a Chromic Luvisol (FAO, Food and Agricultural Organization, 1990) with a description of the soil profile summarised in Table S1. A summary of the rainfall and air temperature over the growth period is given in Fig. 1 (Cfb by the Köppen climate classification). The wheat was rainfed with no irrigation. Three contrasting genotypes of wheat were grown, Maris Widgeon (a heritage long stem variety introduced in 1964), Paragon (a modern elite spring variety introduced in 1999) and KWS Siskin (a modern elite winter variety introduced in 2015). These were grown in experimental plots 9 m × 1.8 m in size, with three levels of N fertilisation and three replicates, comprising nine plots per genotype. Plots were sown on 9th October 2018 and harvested on 2nd September 2019. Soil samples were taken on 17th January to 90 cm in three horizons: 0–23, 23–60 and 60–90 cm; within each block nine cores were taken and bulked, and the total nitrate and ammonium N measured in each layer for each block. The N was extracted with KCl and the extract analysed colorimetrically.

The nitrogen rates were 100 (N100), 200 (N200) and 350 (N350) kg/ha N, applied on three dates (28th February, 3rd and 15th May 2019); the N100 treatment received 50 kg/ha N on the first two dates, none on the third, the N200 treatment received 50 kg/ha N on the first and last date and 100 kg/ha N on the middle date, and the N350 received the same as the N200 except 250 kg/ha N on the middle date. Standard UK wheat husbandry was followed to ensure adequate pest (disease and weed) management as described in Barraclough et al. (2010).

At maturity, the wheat was harvested by combine harvester, with grain yield recorded for each plot. A pre-harvest grab sample was used to measure harvest index for each plot, and straw yield calculated from the grain yield and harvest index (Barraclough et al., 2010). A sample of the harvested grain from each plot was analysed for nitrogen content using a LECO Combustion analyser and the 'Dumas' digestion method. This was then used to estimate grain protein content by multiplying the determined grain nitrogen content by 5.7 (Mariotti et al., 2008).

2.2. Soil sampling and storage

Soil cores of approximately 100 cm long and 9 cm in diameter were collected on 26th June 2019 using a Dando Terrier Rig taking samples in clear plastic liners that were subsequently sealed using plastic caps. Samples were taken c. 100 cm from the edge of each plot to ensure that the region sampled was not affected by edge effects. The cores were transported to the Hounsfield Facility at the University of Nottingham, U.K. where they were stored at 4 °C before X-ray Computed Tomography

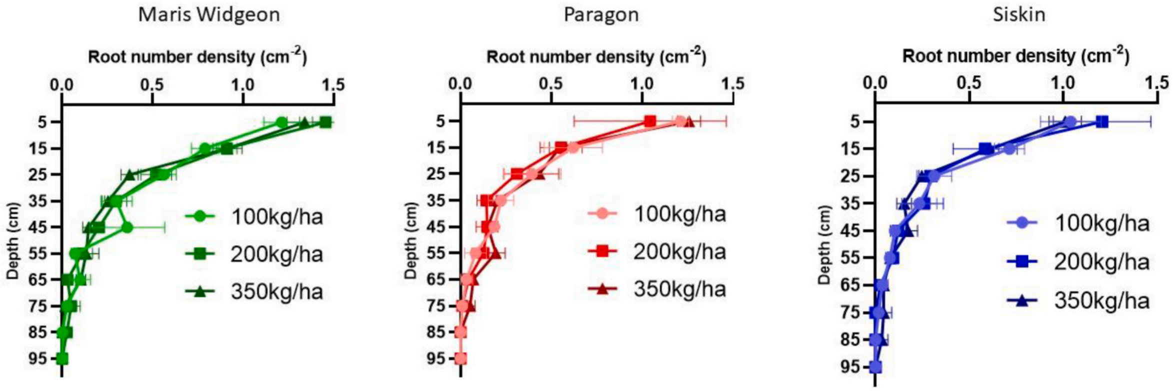

Fig. 3. Root number density up to 95 cm soil depth of the three different genotypes. Error bars represent standard error per treatment at each depth for each genotype.

(CT) scanning and root analysis was carried out on all samples.

2.3. X-ray CT imaging and analysis

The intact soil cores were scanned using a Phoenix v|tome|x L Custom® µCT scanner (GE Sensing and Inspection Technologies, Wunstorf, Germany) using a similar protocol to Zhou et al., (2021). The scans were undertaken at a voltage of 290 kV and current of 2700 µA achieving a spatial resolution of approximately 90 µm. A step rotation of 0.129° per image scan was used over a 360° rotation resulting in 2800 images being taken. For minimisation of image noise, five radiographs were recorded (each taking 200 ms) for each angle with the average being used for image reconstruction. As the cores were much larger than could be accommodated in one scan, eight individual scans were performed for each soil core sample with an overlap of 1 cm between adjacent scans to enable the entire core to be imaged. The different scans were then reconstructed using the multi-scan feature in Phoenix datos x software (GE Sensing and Inspection Technologies) to produce 16-bit 3D volumes of each core. The produced volumes were then imported to VG StudioMAX 3.1 (Volume Graphics GmbH, Germany) and converted to 8-bit tiff images which were saved for subsequent analysis.

The saved images were imported to AVIZO 9.0.1 where a region of interest (ROI) of 600 × 600 pixels (equivalent to 5.4 × 5.4 cm) was selected from the central part of the core to remove potential disturbances from the edges that may have arisen during sampling (NB; no evidence of this was observed). A median 3D filter (with neighbourhood of 3 and 1 iteration) was applied to reduce image noise, then a user-defined global threshold was used to separate solids and pores. The solid material was further separated into the soil matrix and high-density coarse (>2 mm) material referred to hereafter as stones. The segmented ROI was then subdivided into 10 cm sections through the longitudinal length of the core to enable correlation with measurements undertaken at the same intervals for root counting.

2.4. Root counting

The X-ray contrast resolution between roots and soil when embedded in dense subsoils was not sufficient to segment the roots from the soil accurately thus a manual root measurement approach was adopted. The transparent plastic casing which housed the soil was cut lengthwise along the 100 cm of the core using a knife to expose the soil. The exposed core was then cut carefully cut in a single motion, horizontally to the surface of the soil in 10 cm intervals starting at the 5 cm depth (5, 15, 25, 35, 45, 55, 65, 75, 85, and 95 cm) as described by White & Kirkegaard (2010) and Zhou et al., (2021). Each exposed soil surface was then carefully cleaned and the number of visible roots on each exposed surface was counted. The counted roots were then classified as either growing in a macropore/biopore or embedded in the bulk soil. Maximum rooting depth was also noted for each soil core.

2.5. Statistical analysis

The statistical analysis was performed in GraphPad prism v9.0.0. Comparison between means was done either using an ANOVA when data were normally distributed or using the non-parametric Kruskal-Wallis tests.

3. Results

3.1. Grain and straw yield

Grain yield in all the genotypes (Fig. 2A) was significantly ($P < 0.001$) increased by the application of nitrogen fertiliser. In KWS Siskin and Maris Widgeon, the 200 kg N/ha rate gave a significantly higher yield than the 100 kg N/ha, in Paragon and Siskin the 350 kg N/ha rate gave a significant yield increase above 100 kg N/ha but not 200 kg N/ha. There was also a significant difference between the contrasting varieties of wheat, with Siskin producing higher grain yield than the other varieties at all nitrogen rates. The difference between Paragon and Maris Widgeon was variable with the former yielding significantly higher grain yield only at the 350 kg N/ha nitrogen rate.

In terms of straw yield (Fig. 2B), there was a significant difference between the 350 kg N/ha treatment and the 100 kg N/ha for both Paragon and Siskin. There was no response to nitrogen rate in the Maris Widgeon genotype. However, there was a significant difference in straw yield between Maris Widgeon and Siskin at the two lowest nitrogen rates with Maris Widgeon having significantly higher straw yields than Siskin in both treatments. There was no significant difference between Paragon and both the other varieties at all nitrogen treatments.

3.2. Grain protein content

The grain protein content (Fig. 2C) increased with increasing nitrogen application rates in all genotypes. Grain protein content in plots fertilised at 100 kg N/ha was significantly lower than those receiving 200 and 350 kg N/ha. The 350 kg N/ha rate also resulted in consistently higher protein contents as compared to the 200 kg N/ha treatment. In terms of genotypic differences, Siskin consistently exhibited the lowest grain protein content in comparison to the other genotypes at the two higher nitrogen rates. There was also no difference in grain protein content between Paragon and Maris Widgeon at all the nitrogen rates.

3.3. Root number density

Root number density (Fig. 3) generally declined with soil depth with the topsoil (0–30 cm) producing significantly higher root number

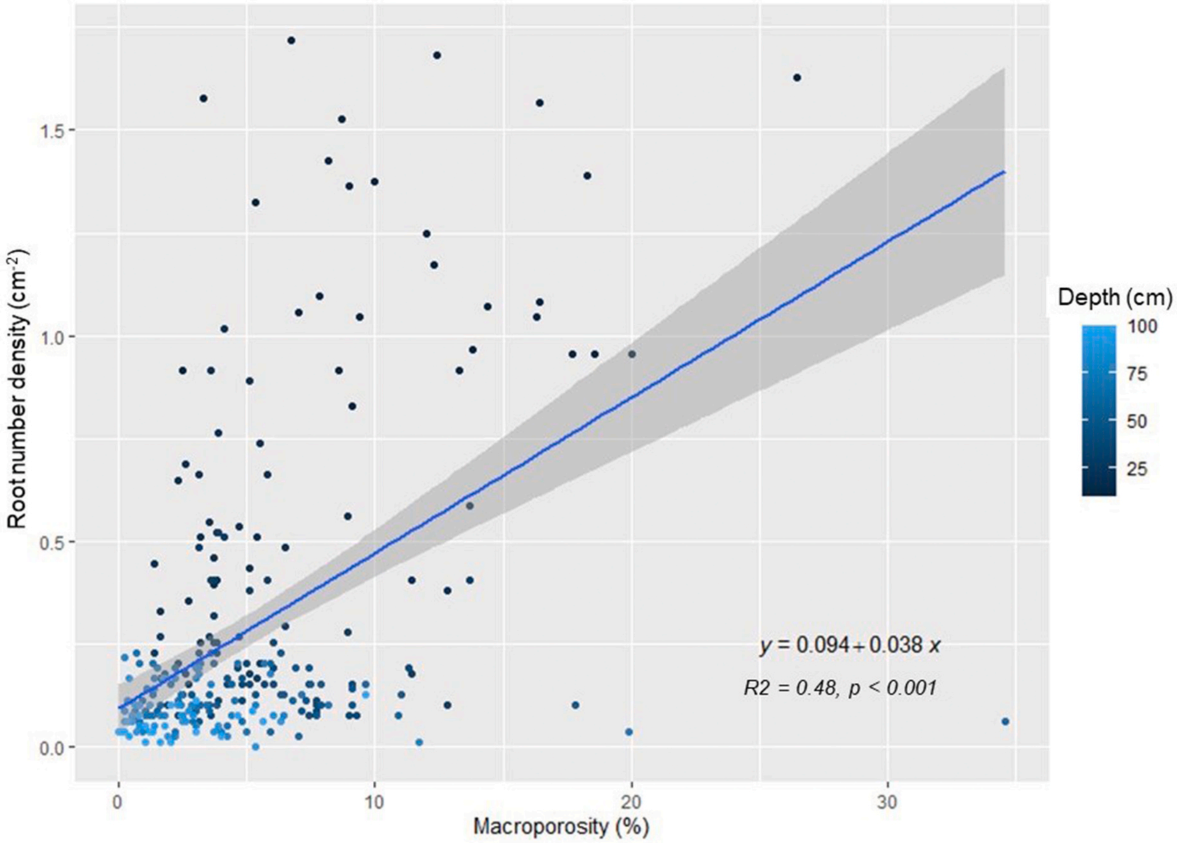

Fig. 4. The relationship between wheat root number density and macroporosity to a depth of 95 cm. Lighter blue colours related to deeper sampling location, and the converse.

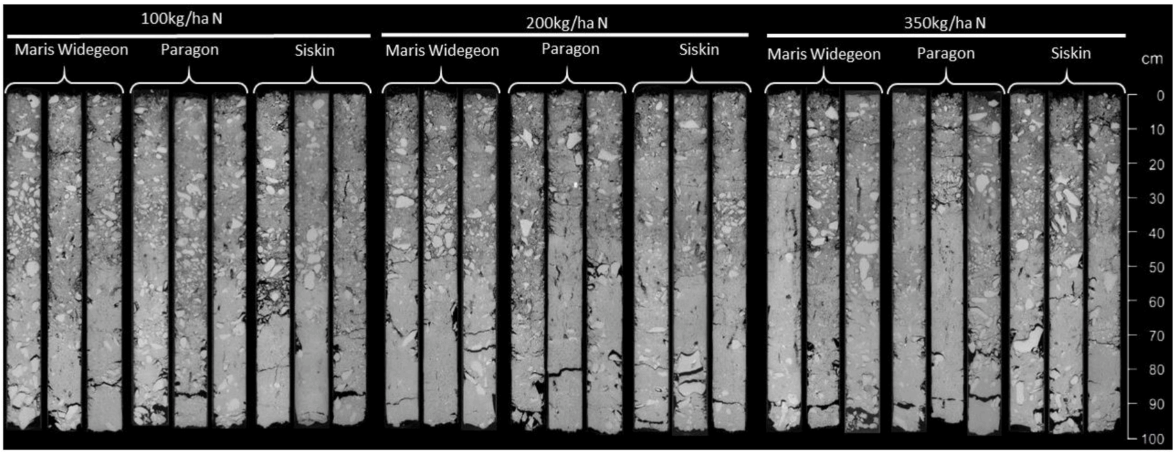

Fig. 5. Longitudinal grey scale X-ray images of the different soil cores from which root trait data was derived. The brighter colours represent high density materials such as stones and some soil material, whilst the darker colours represent low density materials such as soil pores.

density as compared to the subsoil (30–100 cm). There were no significant differences in root number density between the different nitrogen treatments at each depth, nor between the different wheat varieties.

3.4. Soil macroporosity

Soil macroporosity declined significantly with depth, with the highest values occurring closer to the surface of the soil; on average soil microporosity was 18% in the topsoil as compared to 7% in the subsoil (Figs. 4 and 5). There were no significant differences in soil macroporosity between the samples from the contrasting genotypes ($P > 0.05$). Root number density was positively correlated with macroporosity, and a linear regression model identified that macroporosity explained 48% ($P < 0.001$) of the variation in root number density.

3.5. Soil stoniness

The soils in this experiment had significant quantities of stony material which was dominated by flint (a sedimentary quartz mineral rock) with minor inclusions of softer chalky marl material throughout their

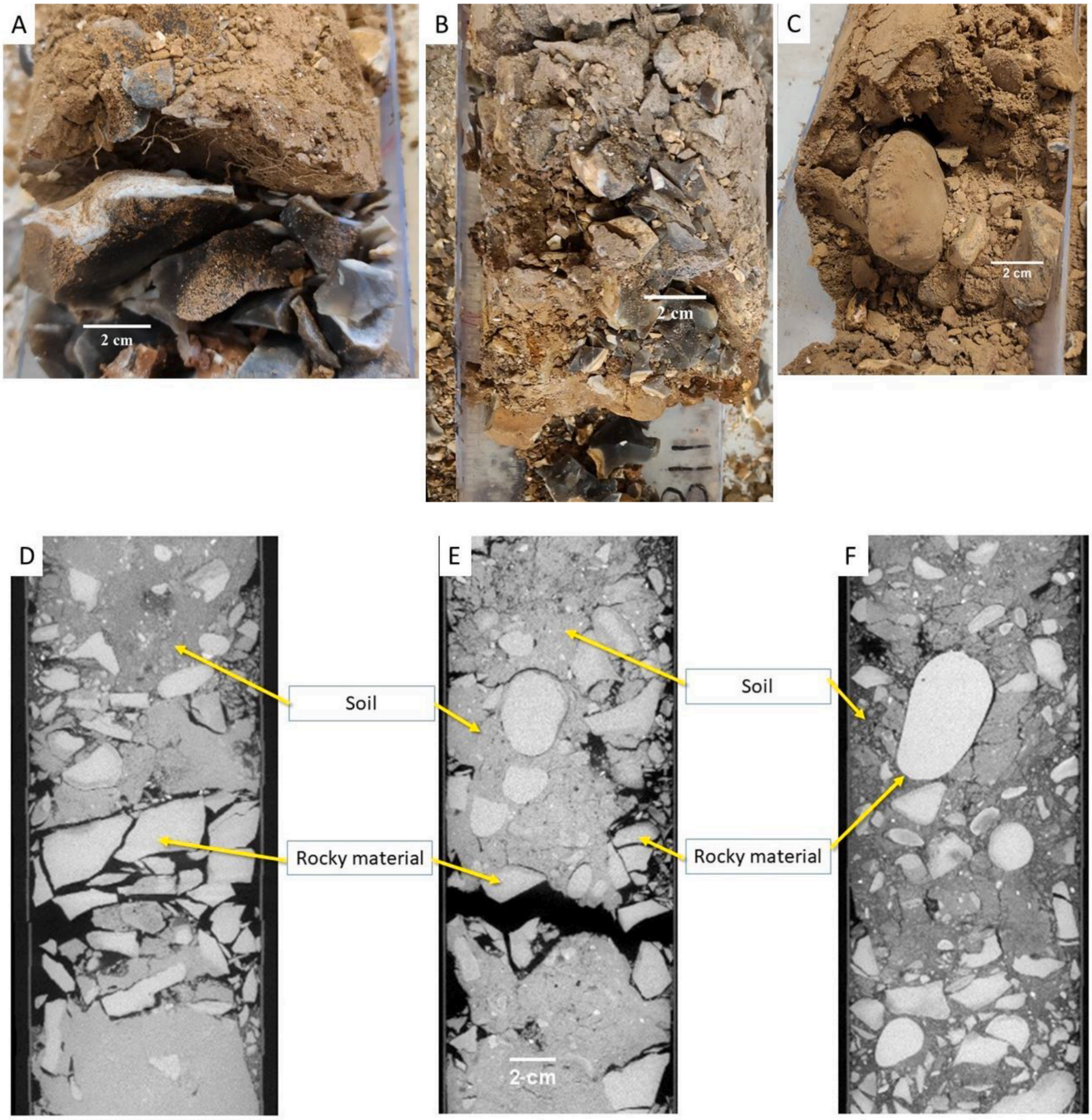

Fig. 6. Examples of the stony/rocky material that was prevalent in many soil cores. A) Shows how root growth may have been hindered by stones parallel to the surface which resulted in mesh-like coiling of the roots above the stones at 30–40 cm depth whilst B) shows the general distribution of stones at 20–30 cm depth of one of the cores. C) Shows distribution of stones in longitudinal form at 40–50 cm depth. D, E and F are longitudinal greyscale X-ray CT scans of the soil columns indicating stony/rocky material (lighter grey) and soil (dark grey).

100 cm profile (Table S1). The soils from Blackhorse Field had on average 17.5% volume of stony material as determined by CT imaging (Fig. 6). There was however significant variation in stony material within each field with some cores having as a little as 6% whilst others had as much as 39% stone content. An increase in stone content with depth also occurred with larger fragments of flint frequently occurring lower than 30 cm in depth. The orientation and distribution of the stones within the different cores sampled also varied between cores with some of the larger stones within the core being horizontally orientated thereby potentially obstructing root growth (Fig. 6A).

3.6. Soil mineral nitrogen

The soil mineral nitrogen varied between 94.3 and 118.3 kg N/ha between the three blocks (Table S2). Approximately 50% of the N was in the plough layer, the 0–23 cm horizon, the rest distributed across the 23–100 cm layer.

4. Discussion

4.1. Response of wheat grain and straw yield to variable nitrogen rates

The three wheat genotypes increased grain and straw yields in response to the higher rates of nitrogen fertilization. As expected, the oldest, long strawed genotype, Maris Widgeon had the lowest grain yield, the highest straw yield and only responded to the 100 kg N/ha treatment. This amount of fertiliser N, plus the soil N, provided enough N for the cultivar to reach full yield potential. In contrast, the most modern and shortest genotype, KWS Siskin (a semi-dwarf line) had the highest grain yield, the lowest straw yield and did respond to increasing levels of N fertilization. Grain protein increased with increasing levels of N fertilization, but was diluted with increasing grain yield, i.e., the concentration of protein decreased across the three genotypes, the lowest yielding having the highest protein. Our results agree with studies that have also reported significant yield increases with

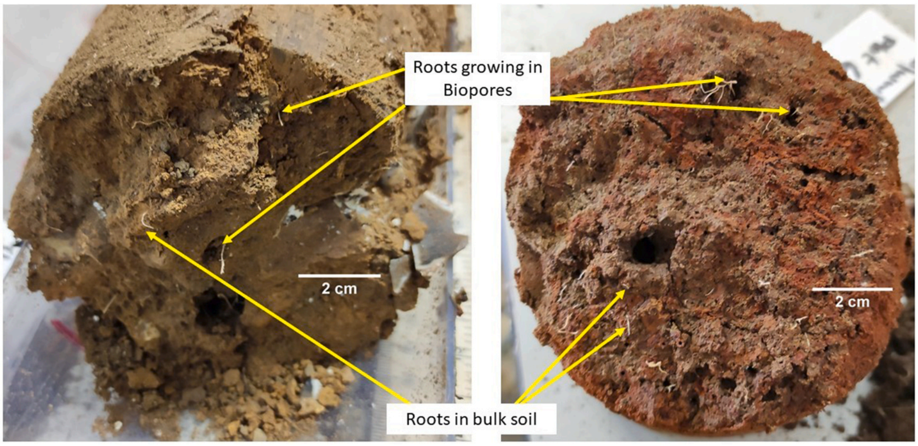

Fig. 7. Example of wheat roots growing in both biopores and in the bulk soil at a depth of 40–50 cm. This rooting pattern was consistent throughout the rooting zone across the samples.

increasing rates of application of nitrogen e.g. Lollato et al. (2019) and Ma et al. (2019). Most of these studies, however, report yield improvements as a result of nitrogen fertilization which often included a control (0 kg N/ha) treatment, which was not included in our experiments. Considering this, the lowest nitrogen application rate (100 kg/ha N) was sufficient to attain near-optimal grain yield in the old genotype, Maris Widgeon, given that further application only increased yield slightly at 200 kg N/ha, and with no significant response to the 350 kg N/ha rate. The highest rate of nitrogen fertilizer application, 350 kg N/ha, would only be expected to give a yield response above the 200 kg N/ha rate when growing modern genotypes in good conditions, but could be expected to result in higher grain protein concentrations, as in our experiment. Ma et al. (2019) and Gauer et al. (1992) showed nitrogen rate was positively linearly related to grain protein even with nitrogen fertilizer rates as high as 350 kg/ha. The lack of a significant yield response between 200 kg N/ha and 350 kg N/ha rate may be due to the dry weather in May–June (2019) (Fig. 1), limiting water and nutrient uptake and thus wheat growth. Such a high nitrogen fertilization rate, however, may come at a cost to nitrogen use efficiency and the environmental considerations, thus a cost-benefit analysis is required to decide the optimal nitrogen rate in any given environment (Hawkesford et al., 2013).

Paragon, an elite spring variety with the dwarfing gene, produced both grain and straw yield that was intermediate between Maris Widgeon and Siskin. Paragon surprisingly exhibited similar straw yields as compared to Maris Widgeon despite being a dwarf variety, whilst in terms of grain, yielded significantly higher than Maris Widgeon. Its superiority in terms of grain yield as compared to Maris Widgeon has previously been reported by Barraclough et al. (2014) in similar conditions. It is noteworthy, however, that despite being a lower-yielding variety, Maris Widgeon often produces grain with superior quality as compared to most dwarf varieties and thus may be desirable depending on the quality of wheat required (Gooding et al., 2012). This was true in this experiment as the grain protein content of Maris Widgeon was higher than Siskin at similar nitrogen application rates.

4.2. *Wheat root response to different rates of nitrogen*

A deep and expansive root system in wheat is considered key to improving yields in different environments (Lynch et al., 2022; Yu et al., 2015). Comparing the root systems of different plant germplasm can help identify unique traits which improve productivity. We found no significant differences between the root number densities within and between the different genotypes at the different nitrogen rates for most of the depths. The only significant differences were at shallower depths (0–20 cm) in Paragon when 200 kg N/ha was applied, increasing root density, as compared to the lowest and highest application rates. Apart from this result, we found no response to increased nitrogen fertilization rates, contrary to several studies that report a localised increase in surface root growth as a result of increased nitrogen application rates (Chen et al., 2020; Gregory, 1994; Lucas et al., 2000). There was also no significant change in root growth in the subsoil in response to increasing nitrogen rates, contrary to what has been reported in the literature where studies have found a significant reduction in root depth with increased nitrogen fertilization e.g. Lucas et al. (2000) and Comfort et al. (1988). Our results however conform to Vincent and Gregory (1989) who reported no impact of nitrogen rate on root growth. Interestingly, genotype did not seem to have an impact on root density and depth distribution despite several reports stating that the modern dwarf genotypes have shallow rooting systems as compared to the older taller genotypes (Aziz et al., 2017; Fang et al., 2017). Our results however concurred with Friedli et al. (2019) and Zhou et al. (2021b) who reported plant root depth and density are not necessarily directly related to plant height.

4.3. *Importance of soil structure and stoniness on root growth*

Soil structure is an important determinant of wheat yield, especially in suboptimal conditions (Bronick and Lal, 2005; Colombi et al., 2017). Our results, similar to Zhou et al. (2021b), reinforced the importance of soil macroporosity for root growth as there was a strong linear correlation between root number density and soil macroporosity. As expected, soil macroporosity decreased with depth, with soil biopores frequently used by roots to grow deeper into the soil. Importantly, contradictory to White and Kirkegaard (2010) and Zhou et al. (2021b), roots were not exclusively found in the identified bio-pores with significant quantities of roots also found in the bulk soil at depth (Fig. 7) which demonstrates the adaptive traits of wheat.

Although stone/rock fragments are ubiquitous in soil, they are most commonly found in the deepest horizons where soil disturbance is typically limited (Zhang et al., 2016). This was not the case in this study as there were considerable quantities of stony material found throughout the samples (though not different between treatments) by volume (Fig. 6). We hypothesize this could have had a negative impact on wheat growth and development, as the stone material regularly exceeded the threshold of 10% identified as being beyond which stones stop being beneficial for plant growth (Grewal et al., 1984; Poesen and Lavee, 1994). This is in agreement with a study by Ercoli et al. (2006) in durum wheat (*Triticum durum.* Desf.) that found stones reduced plant biomass and grain yield in two different durum wheat genotypes. The precise mechanisms by which plant productivity was reduced by stones in our experiments are unclear. In general, stones reduce the soil volume and thus limit water and nutrient availability (Poesen and Lavee, 1994). Furthermore, the effects of nitrogen application in relation to stoniness have received limited attention in the literature. However, we speculate here that stones may have played a key role in determining the depth of growth of wheat roots in response to nitrogen fertilization as stones reduced the effective soil volume, clearly shown in Fig. 6. There are a few studies that have reported plant growth improvements, especially in finer textured soils with <10% stone materials such as Lutz (1952) and Babalola and Lal (1977).

5. Conclusions

Nitrogen rate increased plant grain and straw yields, but not rooting depth. Nitrogen also generally had a limited impact on root number density with only the 200 kg N/ha treatment having a positive effect on the topsoil root number density in one genotype (Paragon). Plant genotype had a significant impact on grain and straw yields but did not affect root growth at all depths. Soil macroporosity decreased with soil depth and was strongly, positively correlated to root number density, demonstrating the importance of soil structure on wheat growth, possibly more so than nitrogen in this case. We also hypothesized the stoniness of the soil could have negatively impacted on root growth. Our results indicated that plant genotype is more important to grain yield than rooting proliferation in response to increasing levels of N fertilization. As such, interventions to improve soil structure such as those measures associated with Conservation Agriculture which are gaining popularity and linked to increased biopore formation may be key to enhancing subsoil rooting and ultimately, grain yield.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the study reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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