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Efect of soil type and amendments on early growth of Kernza Intermediate Wheatgrass

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name "Effect of soil type and amendments on early growth of Kernza Intermediate Wheatgrass" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 19.04.2024 ______________________________

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Summary:

Intermediate wheatgrass (IWG) is a novel perennial crop that can be used for forage and grain production. Recent studies have demonstrated it's potential to provide valuable ecosystem services such as promoting soil health and nutrient cycling. However, research into agronomic practices has been limited especially in the European context. The first part of this study examines existing literature to assess the feasibility of high yielding perennial grains in the near future, as well as the potential of IWG to address critical agroecological issues. The second part describes the experimental portion where we conducted a pot experiment to assess how management practices and soil affected early growth. Namely, how different fertilisers and soil type from various areas affected the young plants. We found soil type affected biomass significantly, and lower drainage capacity was correlated with less growth. Poor growth was also observed in soils with extremely high levels of phosphorus, indicating IWG does not grow well in excess concentration. NPK fertiliser was found to significantly increase early growth, however we were not able to distinguish which nutrient ratio was better or worse. Ongoing research about agronomic practices would benefit from a longer-term field study looking at specific nutrient deficiencies in the soil and how to address them using tailored fertiliser amendments.

Keywords: sustainable agriculture, perennial grain, pot experiment, biomass, soil, fertiliser.

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1 Introduction

Annual grain agriculture and the intensive management associated with it contribute to problems such as accelerated soil degradation, nutrient leaching and increased reliance on herbicides and pesticides (Crews et al. 2018; Reilly C. et al. 2022). Soil degradation is becoming an increasingly pertinent threat to the Czech agricultural sector, due to the prevalence of grain crop monocultures that span large areas of land (Borůvka et al. 2022). In the midst of these challenges, the emergence of perennial grain crops, such as Intermediate Wheatgrass (IWG) or Kernza®, presents a promising opportunity for the radical transformation of these systems (Adebiyi et al. 2016). Kernza®, a dual-purpose forage and grain crop, differs from its annual counterparts in that it provides soil cover year-round and has a deeper rooting system (Duchene et al. 2020). Perennial grains offer growers reduced reliance on synthetic inputs due to increased nitrogen use efficiency, and enhanced ecosystem services (Asbjornsen et al. 2014; Stolarski et al. 2017). However, the feasibility and adoption of perennial grains, particularly in the Czech agricultural context, remain relatively unexplored.

This paper aims to address one of the gaps in understanding the potential of IWG cultivation in Czech agriculture. Through a series of pot experiments, we investigate the influence of soil type and fertilization on the early growth and biomass production of Kernza®. By examining these factors, we seek to provide insights into the feasibility of the adoption and integration of this crop into Czech agricultural systems and provide initial research that may inform future field studies on a larger scale.

2 Scientific hypothesis and aims of the thesis

The objective of this thesis is to compare early growth of Intermediate Wheatgrass (IWG) under different soil types and soil ammendments. A series of pot experiments to grow IWG for several weeks under the same environmental conditions will be set up in order to compare the effect of the treatments. First, an experiment to look at the effect of different soil types, collected from four locations. Second, an experiment to compare fertiliser amendements and effect on above and below ground biomass. Overall, this thesis aims to conduct early agronomic research to determine how early growth can be optimised.

It is expected that IWG final above and below ground biomass will be affected by soil type and composition. Specifically, heavier clay soils are not expected to be suitable for plant establishment. Additionally, plant biomass is likely to be affected by the application of fertilisers. We expect biomass will be increased by NPK fertilisation, specifically nitrogen.

3 Czech agricultural context

Czechia spans an area of 78,871 km^2, of which 53.4% is classified as agricultural land. This amounts to 4.2 million ha made up of 37.5% arable land and 13.0% grassland (Ministry of Agriculture of the Czech Republic 2018). The most commonly grown crops include cereals, oilseeds, pulses and root crops (EUROSTAT 2018). Cereals, especially wheat, maintain a dominant position on the market and winter wheat makes up around 60% of all cereals produced. The total harvested area occupied by cereals in 2016 was 1359 thousand ha and wheat represents 839.7 thousand ha, just over 60% (Štástná et al. 2019). Production in 2016 was 5.455 million tonnes and although wheat is an important export crop, between 2006 and 2016 production was meeting domestic consumption needs (Ministry of Agriculture of the Czech Republic 2018).

3.1 Soil degradation in Czechia

One of the biggest challenges for Czech agriculture is soil degradation (Borůvka et al. 2022). Soil is threatened by processes including erosion, nutrient leaching and features of the changing climate such as prolonged drought. The Czech Ministry of Agriculture recently reported that 60% of agricultural land resources consist of less to poor fertile soils. This is, in part, due to soil water erosion and reduced water retention in soils, which jeopardises 53.9% of agricultural land, as well as wind erosion which threatens up to 45% of agricultural land. The estimated loss of topsoil in Czechia is 21 million tonnes annually, which corresponds to an economic loss of at least CZK 4.3 billion (Ministry of Agriculture of the Czech Republic 2018). On a global scale it is estimated that 24 billion tonnes of soil are lost annually (Montgomery 2007).

Soil degradation is largely exacerbated by the endogenous nature of land ownership and use in the country. The average farm size of 130 hectares is the largest in the EU. Additionally, 80% of all agricultural land is farmed by tenants, which tend to be big agricultural enterprises (Štástná et al. 2019, Sklenicka et al. 2020). These large operations are highly mechanized and Tittonell et al. (2020) note that many are reliant on the use of external inputs necessary for increased production. However, this also comes with a wealth of environmental problems such as habitat disruption, fertilizer and pesticide contamination of soil and water, and the loss of biodiversity on farms and the adjacent natural land that farms expand into (Pe'er et al. 2014; Fischer et al. 2018; Wezel et al. 2018). Large farms spanning vast areas are also more susceptible to soil degradation (Borůvka et al. 2022). The reasons for this are not straightforward, but rather an interaction between land stewardship, farm size and specialization of agricultural systems. D'Souza & Ikerd (1996) found that small farm operators are better stewards of the environment. Boserup (2005) emphasises that operations owned by family members, as opposed to enterprises, had a stronger motivation to protect soil on the farm so that it may be passed on to the next generation. Similarly, a study by (Sklenicka et al. 2020) investigating the relationship between land users and land degradation found farm size to be the most significant predictor.

In Czechia during 2021, the sowing area for winter wheat was 710 thousand hectares (Czech Statistical Office 2021). This area amounts to just over a quarter of all the agricultural land in Czechia. Sowing usually takes place between mid and late October and harvest throughout July (Vrtilek et al. 2016). Even when taking into consideration variability for early and late harvest, the implications are that thousands of hectares become bare within a short period of time. Winter sowing can be especially problematic because of the frequency of intense weather events during crop establishment. Until the young canopy fully develops, soil is vulnerable for weeks after sowing (Crews et al. 2018). Gebeltová et al. (2020) note that cereals tend to be grown in rotations of spring wheat, rapeseed, winter wheat. This high share of cereals in the rotation can lead to imbalanced nutrient removal and soil degradation.

Others argue that the largest contribution to soil degradation is the persistence of annual grain agriculture. Soils that host annual grains are “chronically disturbed” and experience erosion levels which exceed the soil replacement rates. Crews et al. (2016) argue that annual grain ecosystems undergo the greatest disturbance on the planet when areal extent, frequency and intensity are taken into consideration. Chronic disturbance leaves ecosystems in a perpetual state of early succession and diminishes any ecosystem services beyond food production. When managed using conventional tillage, landscapes erode at more than 100 times the rate of soil formation (IPCC et al. 2019).

The Rural Development Programme (RDP) for Czechia has identified combatting soil degradation as one of the top priorities. Priority 4 is dedicated to “restoring, preserving, and enhancing ecosystems related to agriculture and forestry” (European Commission 2022). This includes promoting organic agriculture, supporting the maintenance *and* conversion to organic management. Notably, maintaining sustainable farming in areas with natural constrains (ANCs) is also mentioned. In Czechia, 50% of agricultural land is classified as ANCs - land that is inherently more difficult to farm because of sloped ground, excess soil moisture, poor chemical properties etc. The potential for intermediate wheatgrass to be integrated on marginal land is discussed later in this work.

The rules of organic farming are unique in that they are codified and regulated by certifying institutional bodies (MacCormack 1995). As a farming practice, it relies on diversified crop rotations, prohibition of synthetic agrochemicals and improving soil quality through incorporation of legumes and closed nutrient cycles (Sandhu et al. 2010; Gomiero et al. 2011). Research has demonstrated tangible positive effects on soil quality due to larger inputs of organic matter for fertility and structure, and diverse crop rotations in terms of species and planting time (Erhart & Hartl 2009). Organic farming has the potential to deliver solutions for a wide range of challenges, but this is not always the case. Darnhofer et al. (2010) describe the phenomenon of the rise of “conventionalism” in organic farming. It encapsulates the use of practices that are not necessarily sustainable but are also not expressly prohibited by any rules or standards. A good example of this is conventional tillage practices. In the absence of herbicides, tillage is necessary for weed control. The practice is not prohibited under organic management but many studies have explored the potential soil degrading effects it can have.

De Araújo et al. (2016) studied the effects of different tillage systems under organic management and found higher accumulation of soil organic carbon when using conservation tillage (reduced or no till). Seitz et al. (2019) also found a major improvement to soil erosion when using conservation instead of conventional tillage. Therefore, organic farming, even governed by codified rules, is not a guarantee of “alternativeness”(Guthman 2004). There is evidence to suggest that combining organic farming and practices like conservation tillage have a positive effect on the landscape. Additionally, Adebiyi et al. (2016) found awareness about soil erosion to be positively correlated with adoption of alternative management practices such as no till agriculture, agroforestry and organic farming.

3.2 Rift between crop production and animal husbandry: the rise of specialised systems

Over the past three decades the Czech agricultural system has been subject to many structural changes, initially with the transition to a market economy after the Velvet Revolution in 1989 followed by Czechia joining the European Union (EU) in 2004. A major example of one of these changes is the gradual separation of crop production from animal husbandry and the rise of specialised agricultural systems. Czechia’s accession into the EU failed to stop the growing rift as crop output surpassed livestock output (Věžník et al. 2013). The structure of domestic agriculture changed in favour of more profitable cash and energy crops (Martinát et al. 2013). Between 2000 and 2018 meat production decreased by 36.42% from approximately 703,000 tonnes to 447,000 tonnes (Šrédl et al. 2021) coupled with a two thirds decline in the number of animals (Hlisnikovský et al. 2020). This led to a decrease in the use of manure for fertilization and an increase in mineral fertilizer use for the maintenance of productive operations.

Sekaran et al. (2021) note that specialised intensive agriculture relies heavily on pesticides and fertilizers to ensure a good yield and crop health. In socio-economic terms, the buffer capacity of these farms is low during market price fluctuations (Wezel et al. 2018). By its very nature, the “entrepreneurial” segment of agriculture is dependent upon the markets and institutions that allow intensive farms to grow quickly, whilst accumulating high levels of indebtedness (Van Der Ploeg 2017). Any instability in the market therefore can thus become a major threat to productivity and ultimately food provisioning.

On the other hand, integrated livestock systems can reduce the need for fertilizer input by up to 40% and reduce soil erosion (Kumar et al. 2014). The presence of livestock affects the farmer’s crop choice; when crops grown on the farm can be used as feed, a closed nutrient cycle is achieved (Van Der Ploeg 2017). This diversifies production, which in turn reduces pest and disease pressure and can even improve soil attributes as crops are rotated. By staggering the planting and harvesting of different crops, farmers are able to utilize labour and financial resources better (Macdonald et al. 2013)

In this context, the perennial wheat relative Intermediate Wheatgrass (IWG) presents a radical and transformative alternative. IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, is a deep-rooted, dual-purpose forage and grain producing crop. It is a cool season plant that generally reaches 1-1.5 m in height. Kernza® is the trade name for IWG grain produced by the Land Institute (DeHaan & Ismail 2017). As a perennial, IWG offers year-round soil cover and its ability to improve soil health has been demonstrated in terms of enhanced nutrient cycling, increased carbon sequestration and increased microbial biomass and activity (De Oliveira et al. 2020; Audu et al. 2022; Rakkar et al. 2023). Replacing annual cropping systems with perennial ones that provide feed and food could increase food and ecosystem security and provide an avenue for the diversification of agricultural systems. Integrating IWG could therefore prove to be an interesting consideration for growers looking to reconnect crop production and animal husbandry for a more resilient system.

4 Literature review

4.1 Feasibility of high yielding perennial grain crops

Although perennial grain crops offer a wealth of ecosystem services, long term breeding efforts have always been geared towards improving grain production characteristics to economically viable levels (Bajgain et al. 2023). The initial breeding program of IWG began in the Rodale Research Centre in the 1990s and subsequent long term breeding programs have been established by The Land Institute and the University of Minnesota (Cureton et al. 2023). A close perennial relative of wheat, IWG was initially selected for its promising qualities including vigorous perenniality, seed heads above foliage for harvest purposes, synchronous maturity among others (Bajgain et al. 2023).

In order for IWG to be a commercially successful crop, breeding efforts are focused on increasing grain yield and other agronomic and domestication traits such as free threshing ability, seed size and grain quality and shatter resistance (DeHaan et al. 2020). To date, eight generations of selecting and intermating the best plants based on these desirable characteristics have been carried out. Early on these selection cycles relied on recurrent phenotypic selection and although progress was steady it was not fast enough (Crain et al. 2022). Because perennial crops have a different life cycle to their annual counterparts, it follows that gain (for any trait or quality) per cycle may be similar but gain per unit of time is often substantially lower (Jungers et al. 2023). Challenges for development of perennial species can include the duration of life cycles, 1 to 7 years crop depending, and the need to complete multiple harvests before you the best performing genotype can be ascertained.

In the case of IWG, advances in molecular technology and high-throughput DNA sequencing have allowed for rapid improvement using the power of genomic selection (Bajgain et al. 2023). The breeding cycle has been reduced from three years to one year per cycle (DeHaan et al. 2018). There has been estimated gains of up to 8% year^{-1} for spike yield and grain yield across the eight breeding cycles has steadily increased 9% per cycle (Bajgain et al. 2023). Despite this progress, the grain yield potential of IWG remains lower than that of wheat and produces smaller seed than most annual grains. The thousand kernel weight of naked Kernza® seeds is 7-8 g, compared to 30-40 g for winter wheat (Hayes et al. 2012; Duchene et al. 2019). Researchers hypothesize that another 23 breeding cycles may be required before IWG yields are at a level comparable to annual wheat (Bajgain et al. 2023).

4.1.1 Feasibility of perennial grains

Understandably, the vision of a perennial future has invited criticisms that question whether it is feasible to breed perennial grain crops that maintain perennial vigour and achieve high grain yields simultaneously (Smaje 2015; Cassman & Connor 2022). These criticisms are not unfounded since it has been demonstrated that generally herbaceous perennials tend to have lower seed production than annuals (Vico et al. 2016). Similarly grain yield declines of IWG have been observed after the two-year mark because of nutrient depletion, or the plants becoming sod bound (Tautges et al. 2018).

In response to critics, DeHaan et al (2023) argue in defence of the possibility of significant increases in perennial grain yields. By favouring plants that are less competitive whilst being more stress tolerant, modern selective breeding has reduced competitive waste in annual grain fields. Perennial grain breeders argue that applying a similar methodology should allow similar progress to be made in perennials. Critics of these approaches pronounce the endeavour as unlikely to succeed and unworthy of expanded investment (Cassman & Connor 2022), however, there is increasingly more evidence and research to contradict this claim (DeHaan et al. 2018). Designing cropping systems that somehow mitigate the damaging effects annual crops may have on the soil and ecosystem is not a perfect solution. For example, a no till cropping system that incorporates commercial crops and diverse cover crops – although this is a sustainable approach, the trade-off is perpetual reliance on herbicides which negatively impact human health and the surrounding ecosystem (Crews et al. 2018). Instead of designing annual cropping systems that implement select ecosystem services, more resources should be dedicated to the development of perennial crops which have the potential to address sustainability challenges in agriculture beyond what is possible with annual crops (McGowan et al. 2019; Sprunger et al. 2020; Bajgain et al. 2023)

4.2 Opportunities for soil health and fertility

4.2.1 Nitrate leaching

Integrating perennial crops into agricultural landscapes has the potential to dramatically reduce some of the negative environmental impacts that stem from annual crop production. Specifically within high input grain producing systems, perennialization could address a number of agroecological issues related to soil, including nitrate (NO_{3-}) leaching, soil degradation and soil organic matter loss. One of the most significant effects has been observed with IWG’s ability to prevent nitrate leaching in comparison to annual crops. This is in part due to the extended growing season on IWG, where soil available nitrogen (N) is assimilated at times where it might otherwise be leached, for example when annual crops are absent (Jungers et al. 2019). Kernza® has between 3 and 12 times greater root biomass than wheat (Sprunger et al. 2018a) which is distributed deeper in the soil, which can also help to improve N utilization rate and limit leaching (Ferchaud & Mary 2016).

Culman et al (2013) looked at how short-term leaching of N would differ between IWG and annual wheat under 3 different management systems; organic and two conventional systems with low and high N inputs. An interesting outcome of this study was how quickly after planting any improvements in the soil ecosystem could be detected. This is an important variable if perennial grain systems are to be rotated with other crops. After establishment, lysimeter measurements in the second year showed virtually undetectable NO_{3-} concentrations below IWG: a reduction of 85.8%, 98.2% and 99.4% respectively under each treatment, the highest reduction being seen in the low N plot. Under the high N treatment, the annual wheat plots started showing increased nitrate concentrations and this plot also lost nearly four times as much NO_{3-} compared to the organic annual wheat.

A similar study looking at nitrate leaching losses was conducted by Huddell et al (2023). When comparing NO_{3-} in soil leachate, stand concentrations in the IWG were nearly two orders of magnitude lower than in annual wheat. In addition to leaching the researchers looked at nitrogen use efficiency. More ^{15}N fertilizer was recovered in the soil in IWG compared to annual wheat at all depths. Total soil fertilizer recovery was often higher in IWG whereas total plant recovery of fertilizer was higher in annual wheat. This could be attributed to root biomass of IWG being an order of magnitude higher than wheat (at 1 m depth) and also due to the fact that in wheat, there is a large uptake of N in seeds after heading.

The implications of these studies may be of particular importance in limited nitrogen input systems such as organic or low input systems (Lasisi et al. 2018; Duchene et al. 2019). Perennial grains are most likely to be adopted by farmers who already place importance on conservation farming and ecosystem services as they are less averse to the risk that comes with growing novel grain crops (Adebiyi et al. 2016; Lanker et al. 2020).

4.2.2 Weed control

The commercial varieties of IWG trademarked Kernza® were declared food-grade in the U.S. in 2019 (Bajgain et al. 2020) and more recently in Europe as well. The problem with this is that to date, there are no herbicides approved for use in Kernza® intended for human consumption (Lanker et al. 2020). Although limited, this has prompted research into the potentially weed suppressive characteristics of IWG. Increasing the understanding of these characteristics will prove important for low and high input growers alike, due to the lack of approved herbicides. A study exploring farmer perspectives and experiences growing Kernza® in the U.S. (Lanker et al. 2020) highlighted weed pressure as one of the most pressing issues with growing the novel crop, with calls for more research to be carried out. Perennials impose a unique selection pressure on the landscape (Zimbric et al. 2020) due to the changes in disturbance regime and resource gradient (Fried et al. 2022). With perennial species, there is less soil disturbance and a distinct departure from annual cropping systems, as perennials stay in the soil during periods where there would otherwise be no vegetation i.e. the winter period.

Two recent studies have explored weed suppression and community shifts in IWG (Zimbric et al. 2020; Duchene et al. 2023). Zimbric et al (2020) focused on dual-purpose IWG and the potential effects of forage harvest on weeds. The underlying assumption was that less disturbed ecosystems like grass lands tend to favour grass and perennial species (Adeux et al.

2022). The hypothesis that the weed community would shift from annual to perennials was not rejected. Initially the most abundant weeds were winter annuals until it switched to perennials in the second and third year. They demonstrated the importance of taking into consideration specific characteristics of weed species to predict how they might interact with IWG. Dandelion (*Taraxacum officinale*) was most abundant because it did not get defoliated during forage harvests due to its low growth habit and subsequent ability to make use of more light whilst IWG was regrowing. Forage harvests favour weed species that do not have vertical morphologies with apical meristem above the soil surface. After the second year of growth IWG establishes a dense root system and the researchers used this to explain why plots that received a higher rate of nitrogen had lower weed density when compared to low N plots. They hypothesized that IWG was better at using nitrogen, competing with and decreasing weed abundance.

Zimbic et al (2020) concluded that weed management may not be economically justified because of IWG's innate ability to effectively suppress weeds and maintain productivity in the first year. Although this may have been the case during this experiment, the results contradict other evidence that IWG is a slow growing plant which does not provide ground cover during the establishment year (Jungers et al. 2019; Figure 1). Stem elongation and flowering also happens later compared to annual wheat suggesting weed management could be challenging during this period. The studies that explore weed control in IWG are not comprehensive enough to produce a consensus regarding management. Results and weed emergence will be highly dependent on location, previously grown crop, management systems, weed seed bank, soil type, and climate. This idea was reiterated by farmers describing their experience with weed pressure (Lanker et al. 2020). Those who planted Kernza® on a field with or near an established weed bank found it was most difficult to manage, resulting in poor establishment, a sparse stand and in one case the whole plot was abandoned.

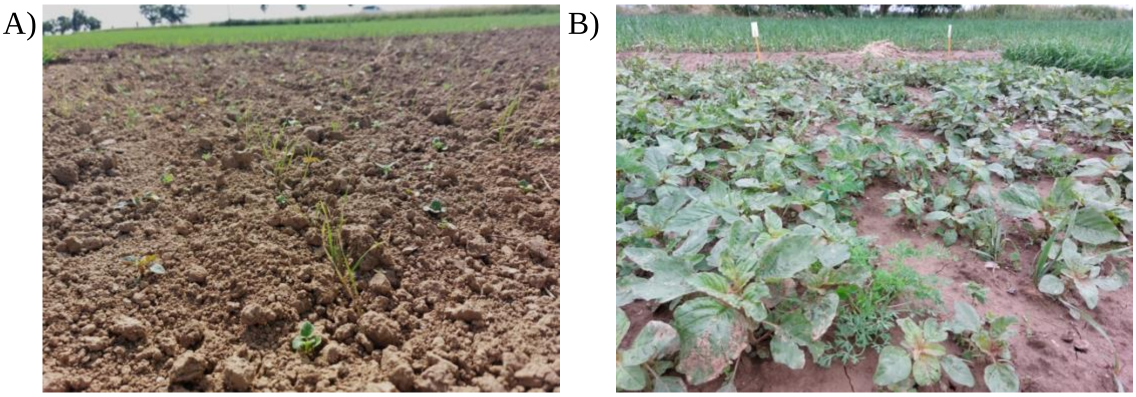

Figure 1. Kernza® variety TLI 703 at A) 3.5 weeks and B) 5.5 weeks after planting in absence of weed control. Picture taken A) June 5, 2023 B) June 19, 2023 by Theresa Pisckáčková, used with permission.

4.3 Market concerns

Developing improved plant material of IWG and increasing grain yield will increase attractiveness for growers. However, it is likely that agronomic improvements may not be sufficient in guaranteeing the commercial success of the crop. Success will depend on the combination of commercialization and plant breeding efforts to develop demand, diversified production systems and facilities in order to create a robust market (DeHaan et al. 2023). In the U.S., Kernza® has enjoyed relative commercial success with companies such as General Mills and Patagonia Provisions developing products derived from Kernza®. The same cannot be said for the European context. Breeding programs have been established in a few select European countries, however the majority of research is still being conducted in the U.S. To date, the EU register of plant reproductive material i.e. seed catalogue, does not list any perennial grains such as Kernza®, severely limiting its use by growers (Duchene et al. 2019)

Jungers at al. (2023) note that as novel crops are introduced, "markets are simultaneously created along with supply chains to make sure new products can be distributed". In some cases, such as perennial rice, the perennial alternative can partially replace its annual counterpart in the market. If the existing infrastructure does not need to be updated, including storage and processing facilities, then commercial success is more likely. However novel crops that undergo *de novo* domestication require different investment of resources to reach the market. This is not a one-way process, often as the market and product are being developed together, market factors can have a strong influence on the crop traits that are selected and improved.

4.4 Marginal integration of intermediate wheatgrass

Having discussed the shortcomings of novel grain crops above, it is important to consider in what context is it likely that Kernza® will be adopted. When asked about integration strategies, potential growers responded that they would have IWG as a "long term perennial crop on sloped or less productive land" and as a "perimeter crop that serves as a buffer" (Wayman et al. 2019). Lanker et al (2019) also noted that IWG is a marginal crop for growers that were interviewed. This is in order to reduce the perceived risk with growing a novel crop with limited information about planting, harvesting and management in general. Areas of the farm that might be difficult to access with agricultural machinery, plots with an irregular shape or plots with poor soil were all identified by farmers as places where they planted Kernza®. Until grain yields are competitive with annual species this is a good way to integrate perennial grains into a system. It is a low-risk solution but solves the problem of empty plots and can demonstrate some of the ecosystem benefits that perennial agriculture offers. Growing IWG on land adjacent to agricultural fields that cannot support an annual crop anymore, allows for land close to skilled farmers to be brought into production (Jungers et al. 2023). The strategic perennialization of marginal lands is also supported by the disproportionate benefits theory, which claims that ecosystem service provision by perennial plants can be magnified, disproportionally to their extent within landscapes (Asbjornsen et al. 2014).

Rakkar et al. (2023) also proposes perennial cropping systems as an alternative intermediate crop for growers transitioning to organic production. The three-year transition period precludes the use of synthetic fertilizers and certain inputs which often reduce crop yields and increase weed and pest pressure. Therefore, using crops which can quickly build soil health, increase nutrient use efficiency and reduce weed pressure is a valuable tool. The study found that integrating IWG could improve soil health more than annuals in the first two years of growth, due to increased root biomass and improved mean wet diameter of wet aggregates in the soil for stabilizing soil structure. Improved water infiltration under IWG also plays an important role in reducing soil erosion under intensive weather events.

4.5 Increasing early growth

Research into methods for improving early growth of IWG will be an important consideration for growers. A perennial grain system offers certain ecosystem benefits to growers: better nutrient use efficiency and less leaching. For these benefits to be realised, optimum fertilizer rates for crop profitability and reduced environmental harm need to be determined (Jungers et al. 2018). Perennial species allocate more resources belowground when compared to their annual counterparts and are often slower to establish than congeneric annuals (Vico et al. 2016). Grain yielding IWG is most productive during the first three years of growth, after which it declines (Jungers et al. 2018). In this instance, starter fertiliser could be important for improving initial establishment and growth and maximising productivity during the first three years.

Starter fertilizers provide immediately accessible nutrients for emerging crop roots, increase the concentration of nutrients that are typically less bioavailable e.g. phosphorus (P) and potassium (K), and improve early season nutrient uptake (Makaza & Khiari 2023). Their efficacy is well documented especially in wheat and maize but research specific to IWG and starter fertilizer is very limited. Once a IWG stand is established, the Kernza® grower's guide recommends autumn manure application at a rate of 90kg N ha^{-1} via surface broadcast for organic production systems (Tautges et al. 2023).

The optimal placement of starter fertilizer also affects overall effectiveness, whether it be banded (subsurface) starter or broadcast. The efficiency of any starter fertilizer placement also highly depends on the soil's physical and chemical characteristics, where soil phosphorus and potassium can play a big role. The importance of placement and soil physiochemical characteristics is illustrated by Parks et al. (1969), who found that broadcast applications of K were 73% as effective than in furrow placement. The K starter fertilizer contributed to reduced lodging of maize and this effect was even more pronounced at low soil K values. One study looking at winter wheat yields observed an increased grain yield when starter N, P and

K fertilizers and potash were broadcast and incorporated. Yield response to broadcast fertilizer was better compared to adding only P pre-planting, followed by a top dress N application (Bushong et al. 2014).

Tailoring fertiliser rates and placement to specific crops is key to reducing nutrient leaching. A study by Ju et al. (Ju et al. 2007) looked at the fate of 15N-labeled urea under a winter wheat-corn rotation. They found when 120kgN.ha^{-1} was applied, 54% was recovered. When 320kg N.ha^{-1} was placed deeper in the soil, only 32% was recovered, despite being almost triple the amount of nitrogen. This problem is what Crews (2005) describes as nutrient asynchrony (Cuevas & Brossard 1994); high input agricultural crops only utilise 30-50% of the N and 45% of the P fertilizer applied. Identifying optimum rates is a delicate balance between over and under fertilising, whilst maintaining yield and profitability. Gourevitch et al. (2018) suggest a definition for socially optimal rates for N fertiliser as “the rate that maximises net benefits of nitrogen to society by accounting for the private benefits and costs of nitrogen”. Perennial crops like IWG interact with soil nutrients differently than annuals. They exhibit higher nutrient use efficiency and can reduce leaching significantly.

Agronomic research about IWG is still sparse, especially in the European context. Most of the research is being conducted in Sweden by the Swedish University of Agricultural Sciences in Uppsala and Lund University (Li et al. 2021; Mårtensson et al. 2022), both research partners of The Land Institute. It is possible to evaluate whether agronomic principles for IWG management developed in other parts of Europe also apply to Czechia. However, before that it is important to conduct preliminary experiments to determine if the crop can successfully become established and grow here.

In this experimental portion of this study we were interested in two research questions: (1) If the early growth of above and below ground biomass of Kernza® is affected by soil type; and (2) If the early growth of above and below ground biomass of Kernza® is increased by fertilisation. In order to explore these objectives, two pot experiments were set up.

5 Experimental design and methods

5.1 Experimental design

Pot trials took place from May to July in 2023 (Figure 2). The first experiment looked at the effect that four different soils had on early growth of the young Kernza® plants (‘Soil type experiment’). The second looked at the effect of five different fertilisers on growth, compared to a control (“Fertiliser trial’). The soil type experiment was replicated in time while fertilisation was not. Both experiments took place in the experimental field at the Czech University of Life Sciences (CULS), Prague (Appendix 1). The pots were covered in a growing pavilion in a semi-controlled environment where it was warm enough but impact from heavy rain was reduced. Therefore, water was supplied though subirrigation and plants were exposed to ambient air temperature outside. During this time, the weather station on the CULS campus showed mean temperatures for May to be 14.3 °C, 18.7 °C in June and 21.3°C in July (Appendix 2). Precipitation data is not relevant and therefore not included.

5.1.1 Germination and planting

As per the latest Kernza® grower guide there are six Kernza® varieties available on the seed market (Tautges et al. 2023). The variety TLI-703 was developed as part of The Land Institute major breeding program and used for both experiments. TLI 703 has a higher number of rhizomes compared to TLI-704 and displays a better performance in drought (Tautges et al. 2023). All the Kernza® seeds were pre-germinated 48 hours before planting, using a seed germinator. Seeds were spread out evenly in a petri dish lined with filter paper and dampened with distilled water. This approach allowed us to select seeds based on germination vigour and uniformity, effectively eliminating, or at least minimizing germination rate as a variable during the remainder of the trials (Allen et al. 1976).

We aimed for the conditions in the pots to be as similar as possible. To achieve this, all visible debris, roots and larger rocks in the bulk soil were removed. We then potted the soil into 11cm square-top pots. This pot size was large enough for the plants to grow for approximately 6 weeks and so experiments were terminated before pot size could become a limiting factor.

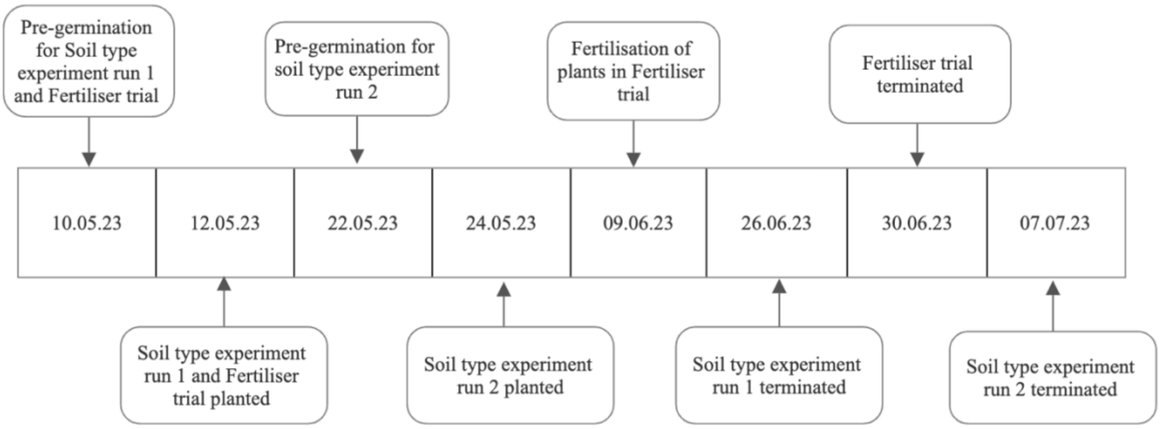

Figure 2. Timeline for germination, planting and termination of both soil type experiments and fertilizer trial. 'Soil type run 1' corresponds to the first soil type experiment and 'Soil type run 2' corresponds to the replicate. Arrows indicate the date of the event and the timeline spans 59 days including the end date.

Figure 3. Set up of Soil type run 2. Picture taken by author on May 24, 2023 after germinated seedlings were transplanted into pots. Soil origin from left to right: a) Štětí b) Pařížov c) Experimental field d) Hořice.

5.1.2 Fertiliser trial set up

The fertiliser trial aimed to test the effect of different fertilisers on early growth of Kernza®. The fertiliser trial included 30 pots, split into groups that were subjected to 6 different treatments. Each fertilizer treatment, as well as the control group, had 5 replicates, totalling 30 pots. In order to isolate fertilisers as the main treatment, the same soil was used for all pots. We used the sandy-loam potting soil from the experimental field at CULS (soil analysis in Table 2). Fertilisation for all plants took place on day 28 of growth and experiment was terminated on day 50. All fertilisers were applied according to the manufacturer's recommendations for 11cm pots (Appendix 3).

The initial 50 days of growing in a pot corresponds to the early growth response that might be observed in the field. Figure 1 shows IWG in the early stages of growth. Figure 1A is 24 days after planting and 1B is 38 days after planting. Between 24 and 38 days is the key period of weed growth during establishment of IWG in the field. Investigating the parameter of fertility in a pot experiment will be a useful indication of what merits investigation under field conditions. Transference of greenhouse results increases appreciably if soil from the same site is used in greenhouse and field experiments (Allen et al. 1976). It is important to note that although the fertilisers were applied at labelled rates, when the experiment was terminated some of the pellets had not fully dissolved, making it difficult to quantify exactly how much fertiliser was released during the growing time.

Table 1. Description of fertiliser treatments applied on day 28. NPK% is fertiliser ratio of nitrogen: phosphorus: potassium and in the case of treatment T4, magnesium as well. N:P ratio is ratio of nitrogen to phosphorus, N:K is ratio of nitrogen to potassium. Form describes the application method for each fertiliser and label rates describes the manufacturer's recommended application rate for an 11cm pot. Each treatment was applied in five replicates.

Treatment	NPK %	N:P ratio	N:K ratio	Form	Label rates
T0	none	none	none	none	none
T1	11-0.2-0.5	55:1	22:1	Pellets	2 pellets/pot
T2	4-2-5	2:1	4:5	Pellets	2 pellets/pot
T3	6-8-7	3:4	~ 1:1	Granules	Granules: 100-150g/m^2
T4	10-5-6 (+2)	2:1	~ 5:3	Pellets	1 pellet/pot
T5	5-10-5	1:2	1:1	Liquid diluted with water	1 cap/3L of water

5.1.3 Soil type experiment set up

Soil was collected from four different agricultural locations in Czechia: farms in Hořice, Štětí, Pařížov and from the experimental field of CULS, Prague (Appendix 1). The four different soils were used for the soil type experiment to explore the potential effects on the early growth of Kernza®. The soils exhibited a range of different pH and nutritional compositions shown in Table 2. Soil type experiment run 1 was set up using the four different soils where each treatment had six replicates, totalling 24 pots. Soil type experiment run 2 repeated this protocol with the same four soils. Soil from the experimental field was treated as a control throughout. Both experiments were terminated 45 days after planting the seeds.

5.1.4 Termination

In order to weigh the above and below ground biomass, upon termination the plants were removed from the pots and the roots were washed. The bulk soil was removed first using a sieve and pouring water gently over the roots, followed by a more thorough wash in a water tray to remove small stones and detritus. After washing, the plants were kept in water to maintain turgor pressure until weighing (Figure 4). The roots were cut away from the leaves just before weighing and we were able to record the measurements for above and below ground biomass, as well as count the number of tillers for each plant.

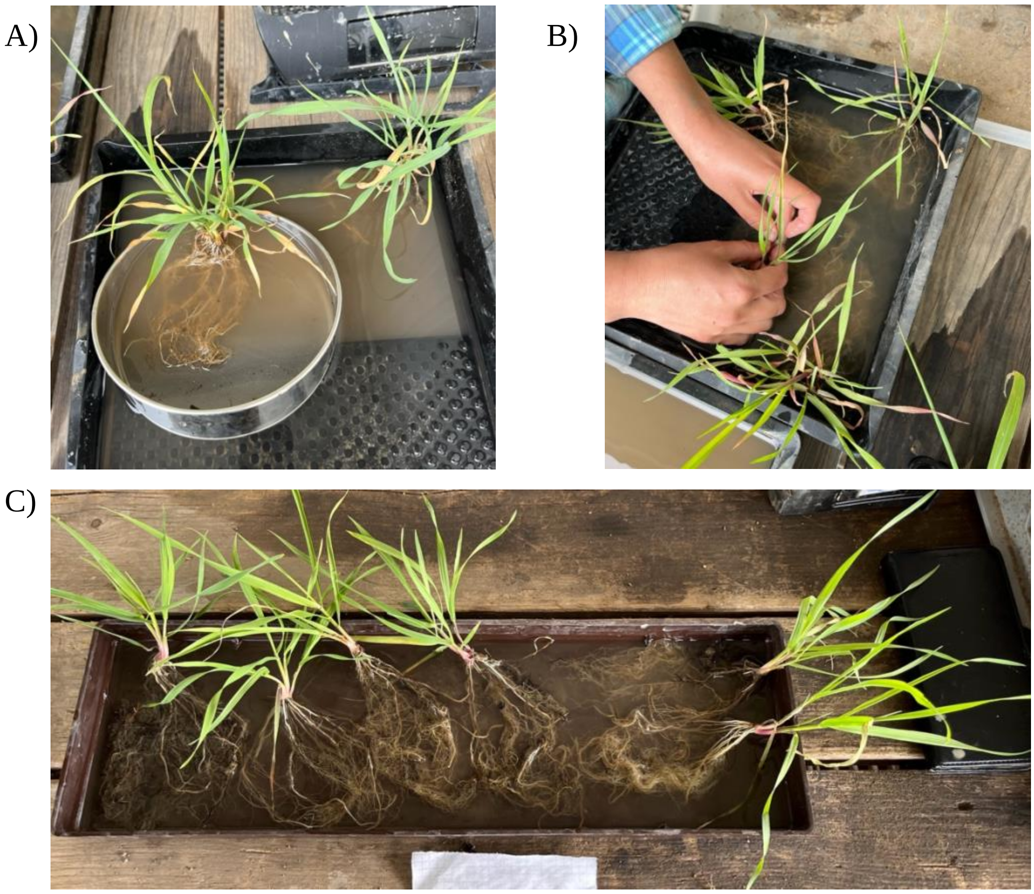

Figure 4. Root washing at several stages: A) Removal of soil from plant roots using sieve and pouring water over. B) Manually removing finer soil clusters. C) Plants in water tray to retain turgor pressure until we could weigh samples. Pictures taken by author on June 30, 2023.

5.1.5 Soil testing

The soil samples from each location were taken to a soil lab where the following standard tests were performed. To determine soil pH and nutrient composition of each soil, two protocols were carried out. For pH determination, soil samples were mixed with a 0.01 mol/L $CaCl_2$ solution at a ratio of 1 part soil to 10 parts solution (weight: volume). The mixture was shaken for 1 hour to ensure solubilization, followed by an hour of standing, allowing any remaining interactions to stabilize. For nutrient content determination soil samples underwent extraction using the Mehlich 3 extraction solution (Mehlich 1984)at a ratio of 1 part soil to 10 parts solution. The soil solution mixture was shaken for 5 minutes and then filtered to separate soil particles. The filtrate was subjected to analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to quantify the concentration of various plant-available nutrients.

5.2 Statistical analysis

Statistical analyses were performed using *R* Studio (R Core Team 2021). The data was analysed using a one-way analysis of variance (ANOVA) to determine if above ground and below ground biomass were affected by different treatments. For the soil type analysis, the two runs of the experiment were not considered as separate data sets, rather they were analysed as one. The analysis of variance of above and below ground biomass data was done separately, however a linear regression was performed to determine the correlation between them for both experiments. Treatment means were then compared using Tukey’s HSD test at $P < 0.05$.

6 Results

6.1 Early growth

The seed germinator method was effective as we were able to obtain the number of germinated seeds we needed to plant in the pots. For the young plants that did not emerge in some pots, we can rule out the seed failing to germinate in the first place as the reason. Emergence of the first leaf after transplanting seeds into pots took an average of 5 days across all the soils. At the end of the experiment looking at soil type the average weight of all plants was 1.35g, above ground average was 0.94g and below ground was 1.76g. The fertilised plants were overall heavier, with an average weight of 3.14g, where above ground biomass average was 2.89g and below ground was 3.40g. In both experiments we observed the roots were heavier than the shoots and that overal the fertilised plants grew more. To assess the relationship between above and below biomass, a linear regression was conducted for each experiment (Figure 5). Based on the R^2 values we can assume that growth of the plants was not limited by the pot size. If the plants had outgrown the pots it would have resulted in a more substantial increase in one biomass component (Wilson BJ 1988). It follows that the results presented should reflect the effect of different treatments that the groups were subject to.

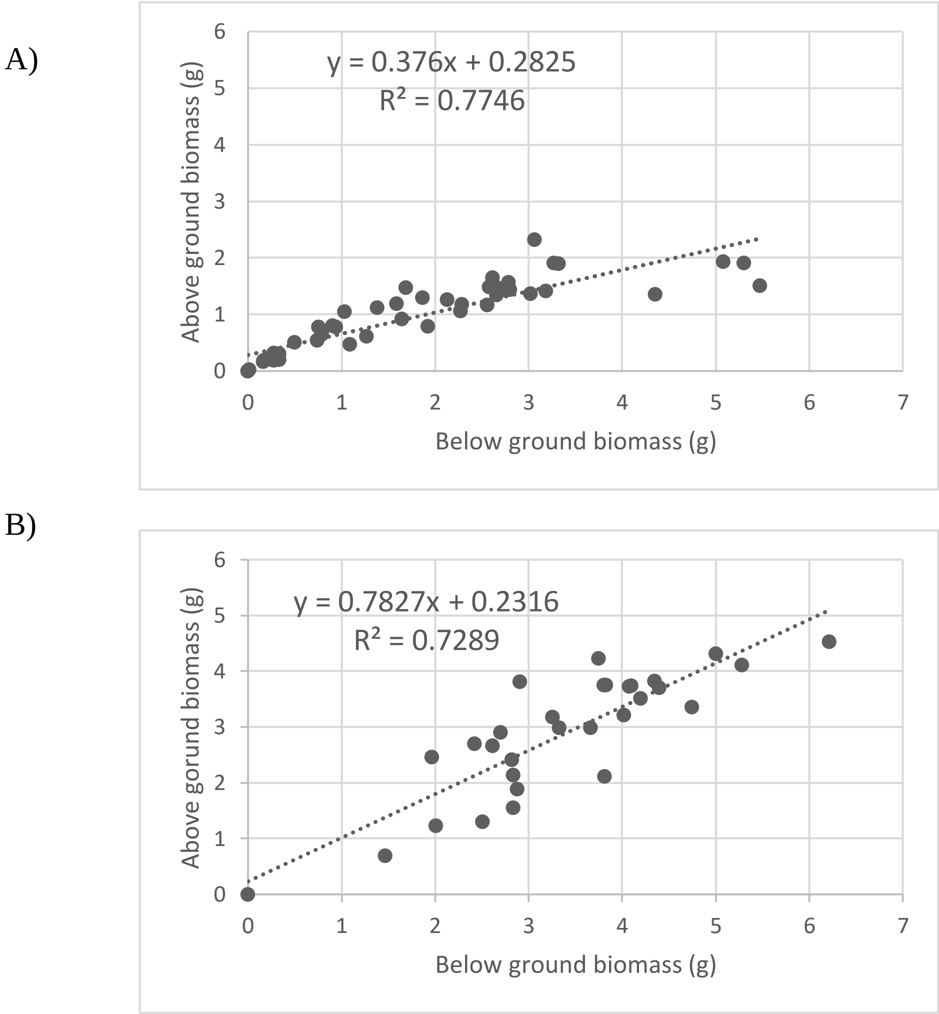

Figure 5. Relationship between above and below ground biomass for A) soil type (n= 46) and B) for fertiliser treatment (n=28). R^2 values describing linear relationship in both trials.

6.2 Effect of soil type

6.2.1 Soil type

Soils represented varying levels of suitability for crop growth (Table 2). The soil analysis highlighted elevated levels of some nutrients in the four soils, as well as some potential deficiencies or limiting nutrients. Štětí and Hořice had a notably low amount of phosphorus, especially compared to Pařížov, which had five times higher P than the satisfactory values. All the soils except Pařižov displayed elevated amounts of zinc. Pařížov also had high amounts of iron and low magnesium.

Table 2. Soil sampling results assessing pH and nutrient availability of four soils. Values marked in red are notably low, values marked in green are notably high compared to the satisfactory values for soils in the first row. For trace elements Ni, As, Pb, Cr and Cd value in first row is not satisfactory values, rather preventative limits for these selected elements. Satisfactory values for Potassium and Magnesium have to be distinguished by soil type; sandy, loamy, clay, so values are in individual rows.

		P	***K***	***Ca***	***Mg***	***S***	***Fe***	***Cu***	***Zn***	***Mn***	***Ni***	***Na***	***Al***	***As***	***Pb***	***Cr***	***Cd***
	pH	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
Satisfactory values		51-80				21-30	61-420	1.61-4.5	2.21-5.0	31-200	4.0			1.0	20	0.74	0.3
Sandy			101-160		81-135												
Loamy			106-170		106-160												
Clay			171-260		121-220												
Hořice	4.9	47.3	202	1360	172	13.0	274	2.06	2.17	100	1.098	27.2	810	0.564	4.08	0.083	not detected
Štětí	6.6	28.2	259	8816	217	17.7	53.2	10.6	38.7	34.9	0.451	47.5	157.2	0.338	5.849	not detected	not detected
Experimental field	6.5	89.6	140.7	3363	137	19.6	137	2.48	8.96	54.5	0.738	33.0	371	0.632	7.37	0.183	not detected
Pařížov	4.8	398	216	1087	99.6	17.8	497	1.80	10.4	59.5	1.953	19.7	968	0.692	5.33	0.175	not detected

6.2.2 Above ground biomass

After 50 days of growing the plants were between 0 – 7.21g across all treatments but had not outgrown the pots. The average number of tillers per plant was 3, the minimum was zero where we observed no growth and the maximum was 8 tillers per plant (Figure 6). Pařížov was the only treatment in which some seeds did not emerge after transferring from the seed germinator (see missing plants and stunted growth Figure 6). Despite all the seeds being pre-germinated, there were still four pots where we recorded zero for above and below ground biomass upon termination. This was unique to this treatment as all other seeds emerged.

Figure 6. Pictures taken upon terminating Run 2 of soil type experiment, after washing roots. Treatment indicated in bottom left corner of picture. Pictures taken by author on July 18, 2023.

The ANOVA results demonstrated that the differences among the groups were considered statistically significant at a high confidence level ($p < 0.001$). These differences were then analysed according to Tukey’s HSD test. When comparing the effects of the four different soils on above ground growth, we found statistically significant differences in biomass. We found Štětí and the experiment field soil were similar to each other and both statistically larger than Pařížov and Hořice (Figure 7). The plants growing in the soil from the experimental field exhibited the highest growth, with an average value of 1.677g across Run 1 and 2 (Figure 7). The plants growing in soil from Pařížov had the lowest growth, with an average value of 0.1255g (Figure 7). Biomass differences were also reflected in the average number of tillers per pot, per treatment; Štětí had an average of 3.83 tillers per pot; Hořice was 2.67; the experiment field was 5 tillers; and Pařížov had an average of 0.667 tillers per pot (Figure 7).

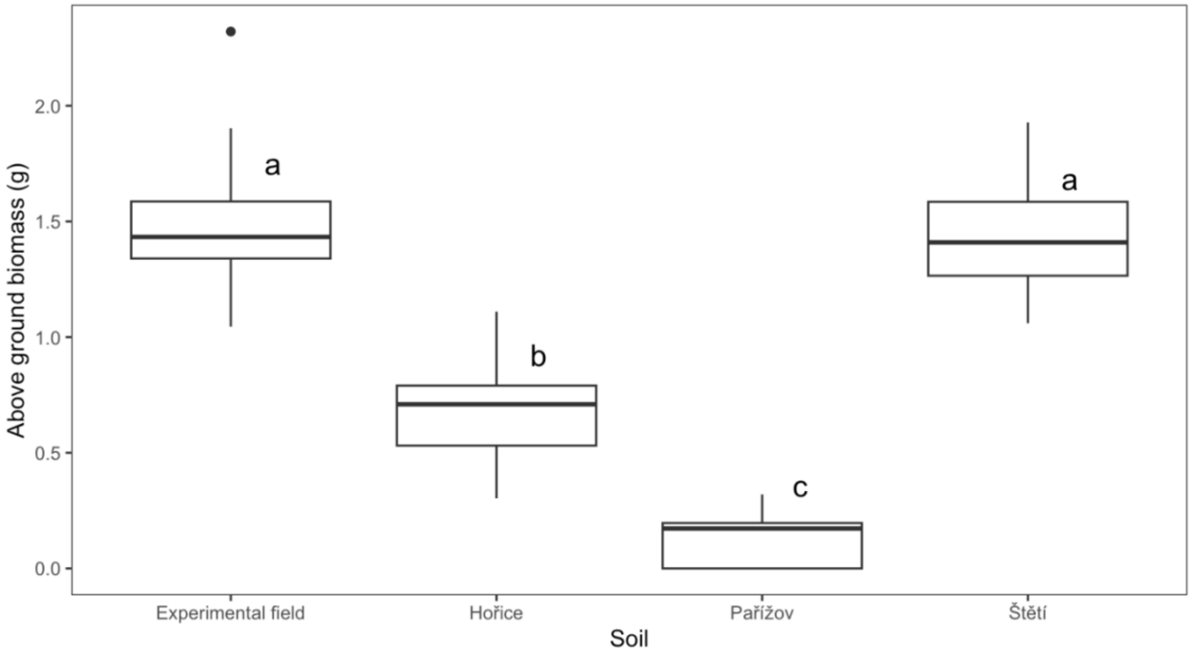

Figure 7. Boxplot of above ground biomass in grams as affected by each treatment. Different lowercase letters represent significant differences between treatments ($P < 0.001$) based on TukeyHSD comparison.

6.2.3 Below ground biomass

The results for below ground biomass followed a similar trend as the results for above ground. There were statistically significant differences between treatments at a high confidence level ($p < 0.001$). In terms of biomass production, the Štětí soil had the highest average across Run 1 and 2, with a value of 3.248g. The lowest was Pařížov with 0.144g, followed by Hořice with 1.026g and the experimental field soil with 2.619g. The roots of the plants of the Štětí treatment were the hardest to clean completely by hand, and we recorded variable percentages (ranging from 2% to 50% of total roots) of detritus that was impossible to remove completely (Figure 8). This could have contributed to the higher biomass average for this treatment.

Figure 8. Roots of plants of Štětí treatment after washing. Darker detritus, observed best in first plant on the left, was impossible to remove during washing without damaging the roots. Picture taken by author on June 26, 2023.

Analysis of differences in mean according to Tukey's HSD test demonstrated significant differences between the treatments at a 95% confidence level. Between them, the experimental field soil and Štětí did not differ, as well as Hořice and Pařížov (Figure 9).

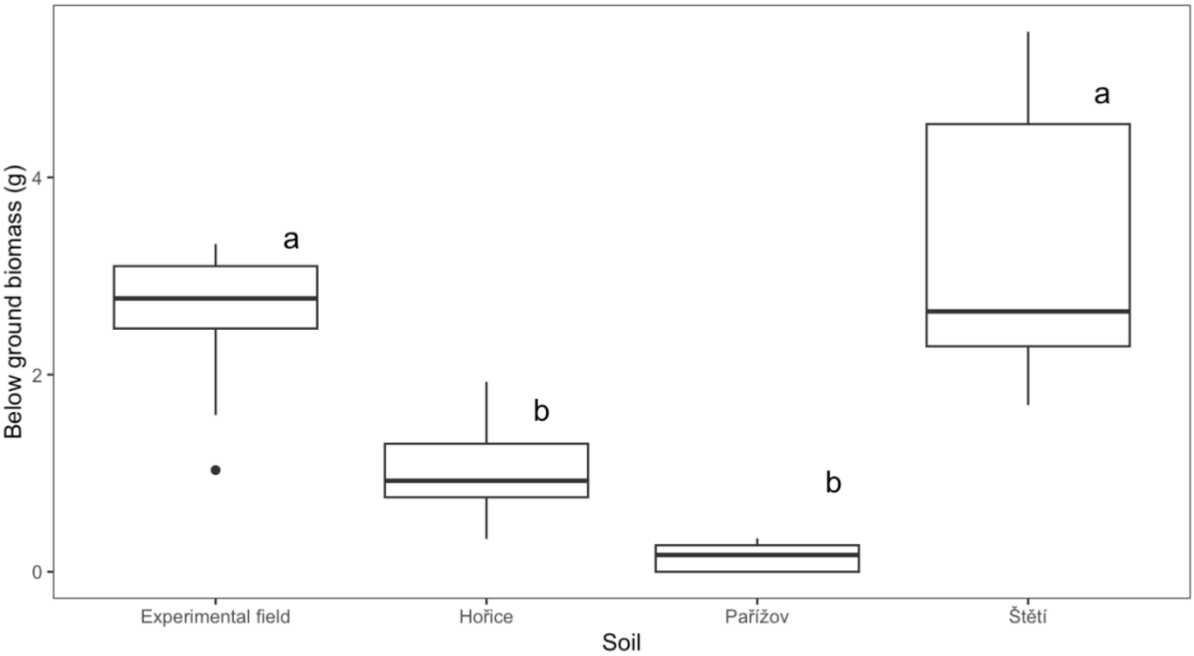

Figure 9. Boxplot of below ground biomass in grams as affected by each treatment. Different lowercase letters represent significant differences between treatments ($P < 0.05$) based on TukeyHSD comparison.

6.2.4 Nutrient analysis

By consulting the Kernza® nutrient deficiency guide published by The Land Institute (Brekalo et al. 2024), we were able to determine whether a specific nutrient was more likely to affect the root or shoot biomass. Using this assumption, we compared data for above and below ground biomass and nutrient concentration in the soil in mg.kg^{-1} and performed a regression to explore the relationship between them (Table 3). Some of this analysis shows a notable correlation between biomass and specific nutrient concentrations in the soil, namely, phosphorus concentrations in relation to above and below ground biomass as well as magensium.

Table 3. R^2 values for regression analysis of either root or shoot biomass and specific nutrients.

Nutrient	*Root/shoot*	*R^2*
K	Root	0.0022
P	Both	Above: 0.6282/ Below: 0.5668
Ca	Both	Above: 0.5169 / Below: 0.7472
S	Shoot	0.1554
Mg	Root	0.5159
Fe	Root	0.1453
Zn	Root	0.4604

6.3 Effect of fertilisers

After fertilising the plants on day 28, the experiment was terminated after a total of 50 days. The plants weighed between 0 and 10.74g and we observed no growth in one of the pots (T5). The average number of tiller per plant was 8.86 and the maximum was 12 tillers per plant. Compared to the soil type experiment, these measurements were higher in weight and average tillers. Upon termination we also recorded the greenness of the leaves and any significant colour changes as this is related to nutrient deficiencies in the soil (Figure 12). In the control treatment T0, most of the older leaves had senesced or exhibited yellowing. We observed that most of the leaves were purpling from the tip to the centre. In T1 the oldest leaves were affected first and were yellowing from the tips to the centre. T2 had a few leaves yellowing at the tips but mostly the older leaves had senesced. Some of the leaves in T3 were purple especially at the tips and on the undersides. T4 demonstrated only some yellowing at the tips of all leaves. Notably T5 had only some yellowing from the tips but no purple leaves.

6.3.1 Above ground biomass

We found statistically significant differences (P < 0.01) amongst the various fertiliser treatments and the control variable where no fertiliser was applied. However, the statistical significance was restricted to differences between the control treatment T0 and fertilizers T1, T2 and T3. Excluding the control, differences in treatment were not considered significant. The highest growth in plants were those under T1 with an average biomass of 3.75g and the lowest growth of fertilised plants were those in T4 achieving 2.78g. In comparison, the control treatment averaged a weight of 1.33g, markedly lower for a short growing period of 50 days. T1 did receive the fertiliser with the highest proportion of nitrogen as well as the highest ratio between N:P (55:1).

We explored the possibility that the form of fertiliser might play a role in growth rate however there is no statistically significant evidence to support this. T3 and T5 received granule and liquid fertiliser respectively, in contrast to T1, T2, and T4 which received the fertiliser in pellet form.

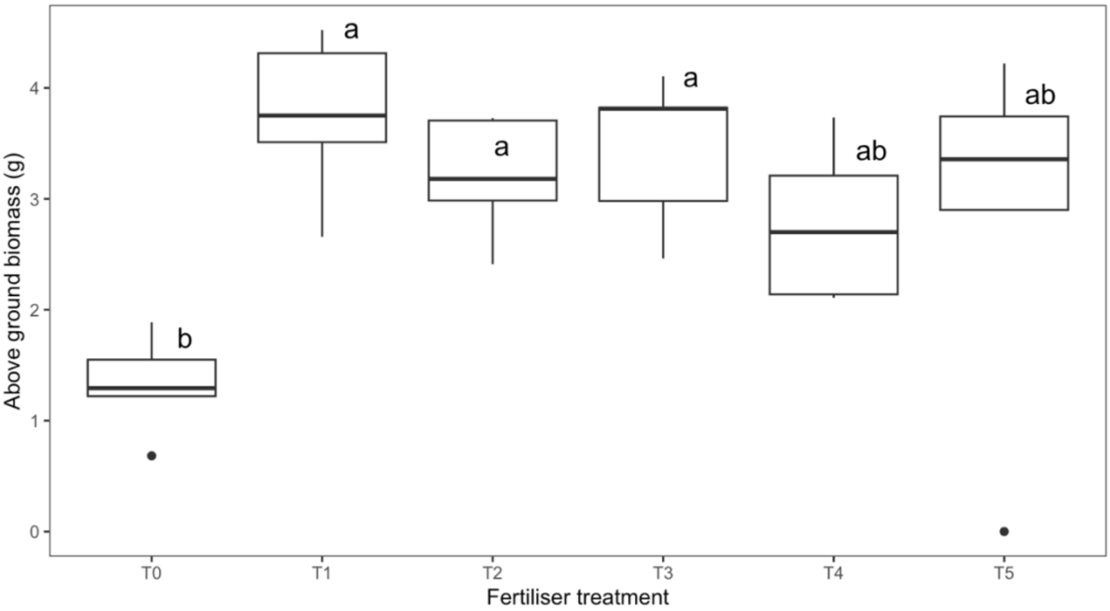

Figure 10. Boxplot of above ground biomass in grams as affected by each fertiliser treatment. Different lowercase letters represent significant differences between treatments (P < 0.05) based on TukeyHSD comparison.

6.3.2 Below ground biomass

Analysis of below ground biomass as affected by fertilisers returned no statistically significant differences (Figure 11). This meant we were not able to show that fertiliser treatments would affect root development. Similar to the above ground biomass, the control treatment exhibited the lowest average biomass at 2.34g and T1 had the highest average at 4.38g, but to reiterate, these differences were not found significant (Figure 11).

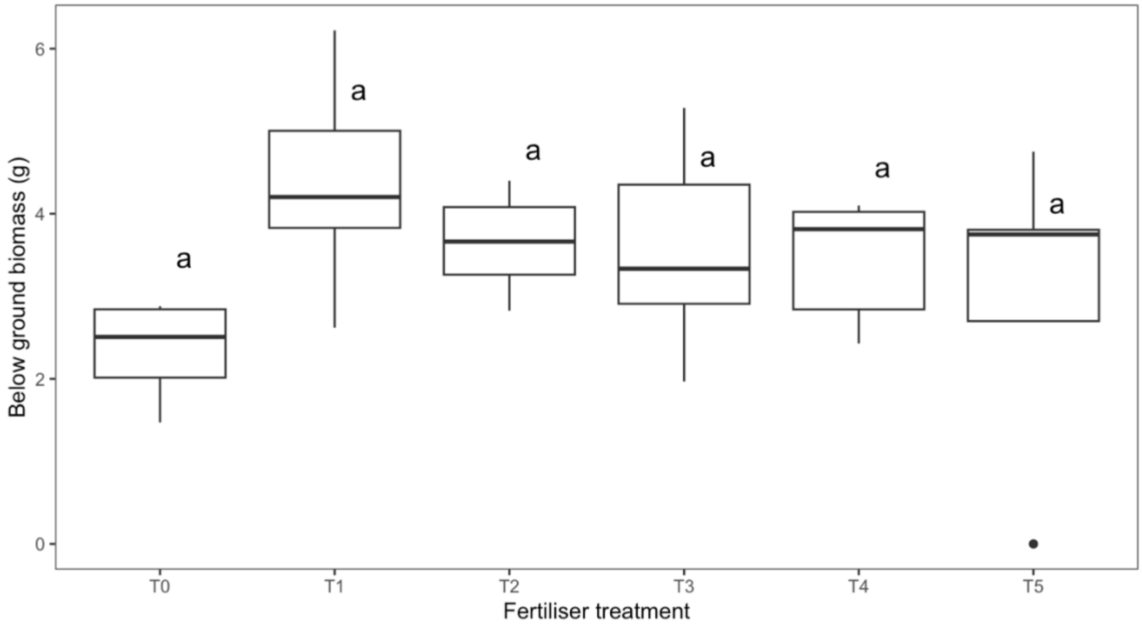

Figure 11. Boxplot of below ground biomass in grams as affected by each fertiliser treatment. Identical lowercase letters represent no significant differences between treatments ($P < 0.05$) based on TukeyHSD comparison.

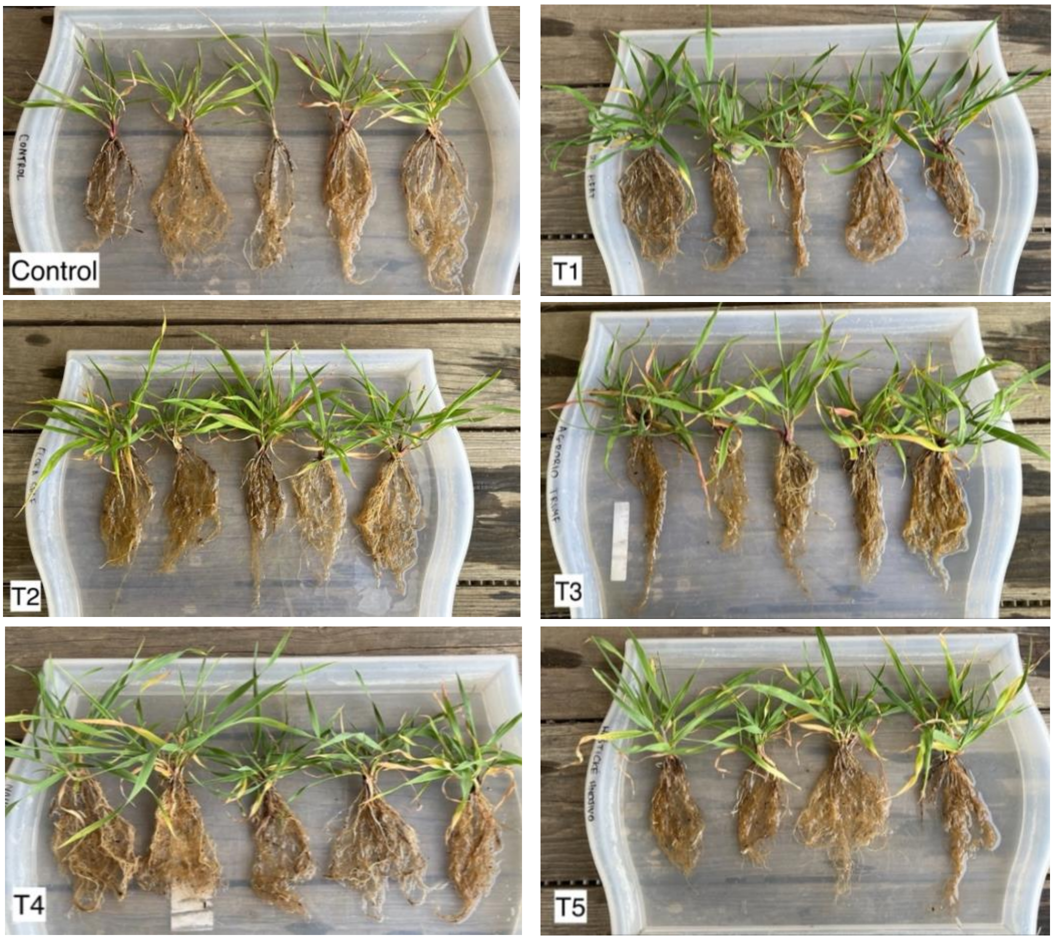

Figure 12. Pictures taken by author on July 18, 2023, upon terminating fertiliser experiment, after washing roots. Treatment number indicated in bottom left corner of every picture.

7 Discussion

7.1 Effects of soil structure on growth

In the soil type experiment, Pařížov and Hořice tended to have the lowest above and below biomass (Figure 7, 9). Both of these soils had properties of clay soil and we expected from the beginning that they would not perform well. When watering the plants, these pots would take the longest to absorb the water, indicating their poor drainage. When the soils were dry they would contract and pull away from the side of the pot. Similarly when setting up the experiment, most of the larger rocks that could have provided a better soil structure for drainage were removed. The Kernza® grower's guide specifies that especially during the establishment year, planting Kernza® in fields that tend to flood, or that have poor drainage, can impact survival – especially in fields with clay soil types (Tautges et al. 2023). The soils that performed better in our experiment, Štětí and the experimental field soil, had a much higher proportion of sand, making it a lot more suitable for the establishment and growth of the Kernza® plants.

In overly sandy soils, nutrient leaching can become a problem, however this must be balanced with Kernza®'s increased nutrient use efficiency, especially nitrogen. Reilly C. et al. (2022) found that on sandy soils, IWG reduced NO_3^- -N in the soil solution by 77-96% compared to annual grain crops. Soil water was also reduced under IWG, suggesting greater water use. The results of our experiment (Figure 6, 7, 9) indicate that, especially during the establishment period, it is important to consider the structure of the soil and drainage capacity when planting Kernza®. Clay soils that exhibit poor drainage capacity will likely jeopardise the establishment of the crop.

7.2 Excess phosphorus

The soil testing procedure revealed that the four soils were most varied in content of phosphorus (P). Pařížov had an extremely high value whilst Hořice and Štětí had very low content. The importance of having sufficient phosphorus in arable soil is well studied in grain crops and perennial grasses (Olszewska et al. 2008; Khyber et al. 2015). Because of its relative immobility, increasing concentration using starter fertilizer can be important for emerging crop roots (Makaza & Khiari 2023). The current recommendations for phosphorus management is soil testing and maintaining soil P, potassium (K) and sulphur (S) amendments similar to those recommended for wheat. The soil test levels of P for Kernza® should be at least 21 ppm Bray-P. Reed et al. (2021) found that for small grains, the 100% sufficiency level is 32.5 ppm M3P. Although the values were obtained using different soil testing methods, Wolf & Baker (1985) note that these two tests are highly correlated ($r^2 = 0.97$). Seeing as the P value for Pařížov was 398 $mg.kg^{-1}$, it is possible that such excess concentration was affecting plant growth.

The linear regression of phosphorus content in soils and root ($r^2 = 0.57$) and shoot biomass ($r^2 = 0.63$) demonstrated moderate explanatory power for variation in the data. The roots of the plants grown in the soil from Pařížov had the lowest biomass in both trials and different morphology from the other treatments. Excess P in soil can reduce primary root length and promote the formation of cluster roots (Williamson et al. 2001). As we observed extremely stunted root systems in the Pařížov - this could suggest that excess phosphorus in soil does not promote root growth in IWG. As discussed below, it is possible that factors such as soil structure played a role in this, however, by looking at the plants in the fertilisation trial, it is possible to confirm that the plants were interacting with soil P. IWG which is P deprived typically experiences a colour change in the leaves to purple (Brekalo et al. 2024). The plants in the control treatment which received no additional P, had many leaves that had turned purple. Out of all the treatments, it had the highest number of purple leaves. The other treatments with varying ratios of P in the fertiliser displayed occasional purpling on the leaves; T1 had leaves purpling from edges, T3 showed it particularly at the tips and undersides of leaves. T5 however, which had the highest percentage of P (5-10-5) and the highest proportion of P:N (2:1), exhibited no purple leaves. In a decade long study, Crews et al. (2022) found no responsiveness of IWG breeding populations to P fertilizer amendments. However, our findings indicate that soil P could be an important factor affecting root growth and biomass development.

7.3 Root development

IWG develops a deep root system that enables it to access water and nutrients more efficiently. Principles of increasing root development are important for the ecosystem services of perennials such as carbon storage and erosion control, which in turn become incentives for adopting the crop. As seen in Figure 11, in the fertiliser trial we were not able to find statistically significant differences in treatments on root growth. This may have been because of the short length of the experiment. Studies have suggested that the exploration of changes in root biomass of perennial grasses is more suited to experiments lasting 3 years and more (Bolinder et al. 2002). Nutrients are cycled seasonally between the roots and shoots (Sainju et al. 2017) and so the true effect of fertiliser on root biomass would be better understood in a long-term study. Sainju et al. (2017) also suggest that root biomass responds more readily to increased precipitation than nitrogen fertilisation.

Once established, perennial crops can have a significant effect on the composition of the weed community and weed suppression in general. Zimbric et al. (2020) emphasise the high levels of below ground competition that the extensive roots of IWG provide. Although we were not able to find a significant difference in root biomass of fertilised plants (Figure 11), the weed competitiveness of IWG is due to its perennial nature, not due to rapid early growth. Rapid early emergence of ruderal and pioneer species can interfere with stand establishment (Figure 1). However, once a stand is established, nutrient use dynamics and light interception both change in favour of IWG and it also provides permanent soil cover, conferring a competitive advantage (Dick et al. 2018; Duchene et al. 2019).

7.4 Nitrogen fertilisation

The need for nitrogen fertilisation of IWG stands has already been recognised (Jungers et al. 2017; Crews et al. 2022). The agronomically optimum rates for grain production proposed by (Jungers et al. 2017) are lower than what is needed to maximize yields of other annual grain crops such as maize, rice and wheat. As fertilisation can impact both below and above ground productivity in a dual use crop, understanding the response to N application will be critical for agronomic management (Vogel et al. 2002). A study by Crews et al (2022) found that when comparing fertilised and unfertilised stands of IWG with different sowing densities, the latter stand exhibited a persistent N limitation across all 5 years of the experiment, regardless of density. Our findings were consistent with this study, inferring that fertilisers containing N increased above ground biomass (Figure 10). As there was no statistically significant difference between the fertilisers, it was not possible to determine which fertiliser composition or nutrient ratio could be most effective.

The currently available studies on IWG do not mention the significance of starter fertiliser. The emphasis on fertility treatments is typically broached after the first year of growth, since IWG grain yields decline after second year. Current recommendations include fertilisation after the first year to mitigate yield decline (Jungers et al. 2017) or fertilising IWG just before the active tillering phase (Tautges et al. 2023). Brekalo et al. (2024) point out that in wheat, effects of nitrogen deficiency early on in growth cycle are not limiting but become so closer to seed production. It is important to note that in our fertiliser trial, we observed a significant difference between the control and fertility treatments within 7 weeks (Figure 10). This should be a consideration for grower’s looking to integrate IWG for a shorter time in crop rotations. This time period also coincides with the critical period of weed emergence for IWG (Figure 1). Banded application of fertiliser could allow for increased leaf and tiller production which in turn would allow for early shading of weeds in the inter-row spaces. High fertility amendments could increase weed growth as well as crop growth. However, Little et al. (2021) note that broadcast applications of fertiliser are more likely to increase growth comapred to banded or deep fertiliser (DiTomaso 1995). If we are able to increase early growth of IWG using starter fertiliser amendments, the crop may have a more competitive advantage for establishment.

8 Conclusion

- In this study we found that the early growth of above and below ground biomass of Intermediate Wheatgrass is affected by soil type and composition. Phosphorus was isolated as a potentially significant nutrient in the soil that could impact root development negatively. This should be a consideration for grower's looking to integrate the crop into marginal fields that have a history of low productivity or poor soil composition.
- Fertilisation increased above ground biomass, however there was no significant difference between fertiliser NPK compositions. Root biomass is likely to respond more readily to other factors such as precipitation which we did not explore in this study. A longer-term study would be needed to explore the possibility of tailoring fertiliser rates and placement to maximise early growth in IWG. The significant response to nitrogen fertilisation of the young plants speaks to IWG's ability to use nitrogen quickly and efficiently in the soil to increase growth.
- We found notable correlation between some specific soil nutrients and above or below ground biomass, however the study of the effect of fertilisers was inconclusive in terms of root growth. Therefore, we were not able to make clear fertilisation recommendations. A potential future study could be centered around testing soils with specific nutrient deficiencies and seeing if fertiliser can compensate.
- IWG is still a very novel crop, especially in the European context. Adoption of it will become more attractive as grain yields of Kernza® increase throughout the breeding cycles and as more research is published regarding agronomic practices. IWG has great potential to be integrated in marginal land and "areas with natural constraints" in Czech fields, however more research would need to be carried out regarding limiting nutrients in the soil. The basis of a following study could be indentidying individual nutrient deficiencies in soil and seeing if specific fertilisers can compensate for the limiting nutrient.

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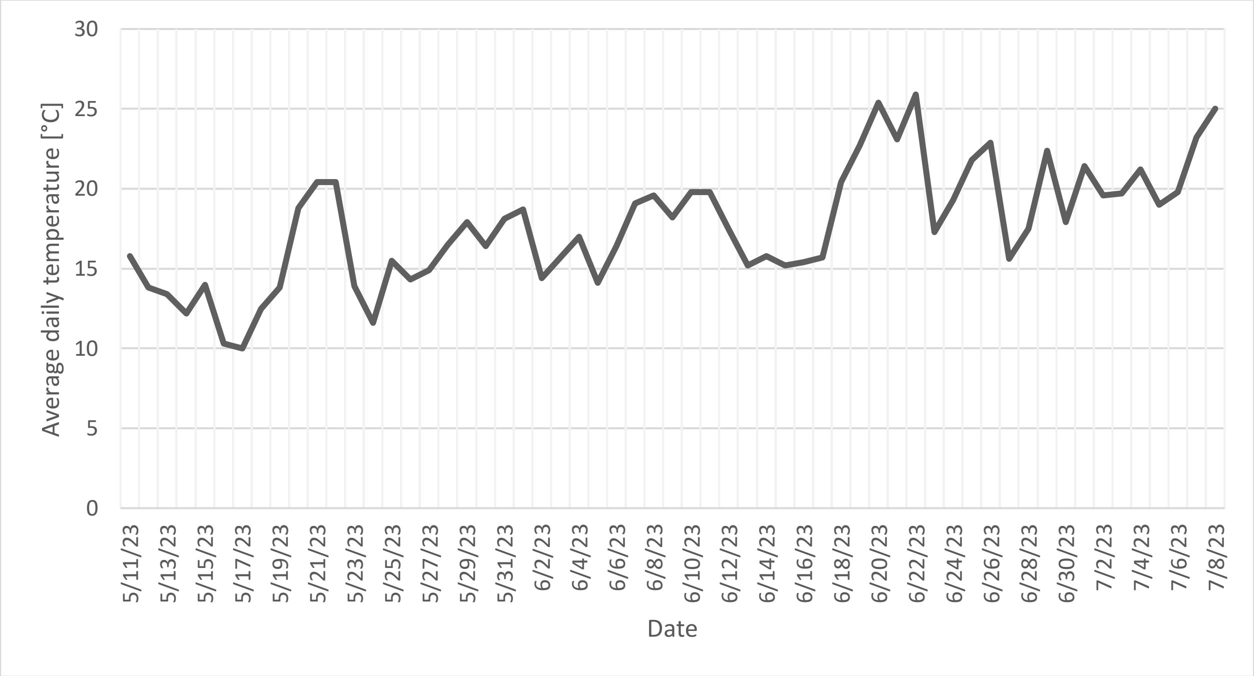
10 Appendices

Appendix 1. Map of Czechia with pinned locations showing agricultural land where soils were collected for soil type experiment.

Appendix 2. Average daily temperature in °C during both soil type and fertiliser trials.



Appendix 3. Commercial names of fertilisers and desciptions of chemical properties as stated on the packaging.

Commercial name of fertiliser	**Treatment name in fertilisation experiment**	**Description**	**Chemical properties**
BigBeat	T1	Long-acting organo-mineral pellet made from plant residues and long-term effective nitrogen fertiliser. Nutrients from organic substances of plant origin enable gradual release. Part of the urea nitrogen is in the form of urea-formaldehyde and part is bound in the organic matter of the plant parts. The fertiliser also contains mineral nitrogen.	Total nitrogen as N: minimum 11%, total phosphorus as P_2O_5: minimum 0.2%, total potassium as K_2O: minimum 0.5%, combustible substances in dry form: 70% and moisture: maximum 15%.
FloraSelf	T2	100% fertiliser with active substances from algae, guano and humic acids. Use instructions follow those outlined above for pellets and apply to all pellet fertilisers used in experiment. It contains plant substances from the production of food and animal feed (wine yeast, cocoa bean shells, dried grain pomace), plant substances from the production of energy (dried digestate), guano, algae and humic acids.	4.0% total nitrogen (N), 3.4% organically bound nitrogen, 0.88% available nitrogen (N, soluble in $CaCl_2$); 2.0% total phosphate (P_2O_5); 5.0% total potassium oxide (K_2O).
Agrobio Turf	T3	Organic fertiliser in granulated form. Mainly made from bone meal, feather meal, blood meal, cocoa husks and vinasse.	Humidity: maximum 10%, combustible substances in dried sample: minimum 40%, total N in the dried sample: minimum 6%, total phosphorus (P_2O_5) minimum 8%, total potassium as K_2O:

			minimum 7%. Fertiliser pH value is between 5.5-7.5.
Nohel Garden	T4	“Universal” fertiliser pellets with little additional information provided. Fertiliser helps to create a crumbly soil structure, this improving physical properties of the soil.	Total nitrogen (urea) 10%, total phosphorus oxide 5% (4% as P_2O_5 and 1% as soluble in ammonium citrate), 6% water soluble potassium oxide (P_2O) and 2% magnesium oxide (MgO).
Hoštické hnojivo	T5	Liquid organomineral fertiliser, main component is fermented chicken manure. Advertised as significantly activating the soil micro flora. High content of phosphorus should be noted as well.	Total nitrogen as N: 5%, total phosphorus (P_2O_5): 10%, potassium (K_2O): 5%, combustible substances in dry form: minimum 50%. Fertiliser pH value is between 4.5-6.5