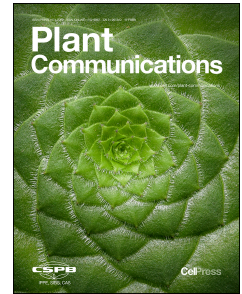


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Challenges facing sustainable protein production: Opportunities for cereals

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## 1 Challenges facing sustainable protein production: Opportunities for cereals

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17

### 18 Short summary:

19 The nutritional quality of food is a rising challenge, with global demand for protein production  
20 to double by 2050. Cereals contribute a major proportion of global caloric and protein needs,  
21 and they provide a more environmentally friendly option for sustainable protein production  
22 than livestock. With new knowledge about the role that altered plant vasculature and  
23 senescence partakes in determining grain protein levels, there are new opportunities for  
24 cereals to significantly impact overall protein supply.

### 25 Abstract:

26 Rising demands for protein across the world are likely to increase livestock  
27 production, as meat provides ~40% of dietary protein. This will come at significant  
28 environmental expense; therefore, a shift towards plant-based protein sources would provide  
29 major benefits. While legumes provide substantial plant-based proteins, cereals are the  
30 major constituents of global foods with wheat alone accounting for 15–20% of the required  
31 protein intake. Improving protein content in wheat is limited by phenotyping challenges, lack

32 of genetic potential of modern germplasms, negative yield trade-off, and the environmental  
33 cost of nitrogen fertilisers. Presenting wheat as a case study, we discuss how increasing  
34 protein content in cereals through a revised breeding strategy combined with robust  
35 phenotyping can ensure a sustainable protein supply while minimising the environmental  
36 impact of nitrogen fertiliser.

37 **Key words:** Sustainability; Protein nutrition; Grain protein content; Innovative breeding

## 38 **The role of adequate protein nutrition in human diet**

39 Food security is central to the sustainable development goals and plenty of research  
40 is focused on providing an adequate food supply for the ever-increasing world population  
41 while protecting the environment. However, ensuring food security does not simply involve  
42 increasing the overall food production, as maintaining a balanced nutritious diet that  
43 accounts for various essential micro- and macro-nutrients is equally important. One essential  
44 macronutrient is nitrogen (N), which animals need to synthesise protein for growth and  
45 repair. Proteins provide 4 kcal energy per gram and are made of chains of amino acids  
46 which can be broadly defined as essential or non-essential, with essential amino acids being  
47 those that our bodies cannot produce and need to be obtained from our diet. Only 21 amino  
48 acids out of roughly 500 identified in nature make up proteins in the human body, with  
49 methionine, histidine, valine, leucine, isoleucine, phenylalanine, threonine, lysine, and  
50 tryptophan considered essential (**Hou et al., 2015**). Of these essential amino acids, valine,  
51 leucine, and isoleucine play a vital role in protein synthesis, energy production, and  
52 metabolic signalling (**Holeček, 2018**). Some amino acids like cysteine, tyrosine, arginine,  
53 and proline are referred to as conditionally non-essential as their synthesis is limited under  
54 pathophysiological conditions (**Lopez and Mohiuddin, 2021**).

55 A deficiency of protein in our diet can result in protein-energy undernutrition and  
56 range from subclinical deficiencies in the form of stunted growth, underweight for age in  
57 children, hair loss, skin atrophy or edema (**Waterlow, 1973**). Protein deficiency has  
58 repeatedly been referred to as a primary cause of child malnutrition, particularly in  
59 developing countries, and malnutrition causes 50% of child deaths under the age of 5 in the  
60 developing world (**Black et al., 2003**). The recommended daily protein intake ranges from  
61 0.75 to 1.6 g per kg of bodyweight depending on age and gender. The quality of ingested  
62 protein is also vital as poor-quality protein can accelerate muscle decline and ageing  
63 (**Levine et al., 2014**). Therefore, adequate protein intake is essential to healthy living.  
64 Recent reviews have discussed future protein supply and demand challenges (**Aiking, 2011**;  
65 **Henchion et al., 2017**), supply of animal-derived proteins (**Boland et al., 2013**), supply of

66 high-quality protein (**Minocha et al., 2019; Wu et al., 2014**), and alternative protein sources  
67 (**de Souza-Vilela et al., 2019; Ismail et al., 2020; Wang and Xiong, 2019**). Here, we will  
68 briefly talk about the major challenges currently facing protein nutrition.

### 69 **Is protein nutrition a challenge?**

70 In 2017, the protein demand for the 7.3 billion world population was estimated at 202  
71 million tonnes per annum (**Henchion et al., 2017**). A study based on FAO data between  
72 1961 and 2011 presented 3 scenarios on the projections of protein demand for the  
73 estimated world population of 9.6 billion (current projection is 9.8 billion) by 2050. All 3  
74 scenarios assumed maintaining the current per capita protein consumption rates for the then  
75 7.3 billion population while making different assumptions for the estimated additional 2.3  
76 billion people. **Scenario 1** assumed protein demand for the additional population at the rate  
77 consumed by developing countries, as the UN estimates that population growth will come  
78 from the developing world (**UNDESA, 2019**), **Scenario 2** assumed protein would be  
79 consumed at the average global rate, and **Scenario 3** at the rate consumed in developed  
80 countries, as the developing countries have shown a significant increase in per capita  
81 consumption in recent decades (**FAO, 2022**). All 3 scenarios estimated an increased  
82 demand of 32%, 33% and 43%, respectively (**Henchion et al., 2017**). Other sources have  
83 indicated an estimated increase of 57% in the global protein demand by 2050  
84 (**Alexandratos and Bruinsma, 2012**). To fulfil a 50% increase in protein demand, intensive  
85 livestock and arable farming is expected, as they contribute more than 99% of the global  
86 protein supply.

### 87 **Challenges associated with animal-based protein sources**

88 Historically, the major sources of protein nutrition are animal- (e.g., eggs, dairy  
89 products, meats, and fish) and plant-based (e.g., soybean, chickpeas, lentils, broad beans,  
90 cereals) commodities, with alternative sources such as insects, algae and aquaculture  
91 gaining attention more recently. Desirable nutritional attributes of animal-based proteins  
92 include the high ratio of essential to non-essential amino acids and balanced essential amino  
93 acid profile (**WHO, 2007**). In contrast, many plant-based protein sources have a low ratio of  
94 essential to non-essential amino acids and poor profile of essential amino acids. For  
95 example, lysine content in cooked chicken breast can be up to 2.7% whereas in wheat it is  
96 about 0.3%. Plant- and animal-based foods provide ~65% and ~35% of protein in human  
97 diets, respectively. Various animal-based sources range in protein content from 14 to 32%  
98 whereas plant-based sources range from 5 to 30% (**Table 1**). In developed countries, the  
99 consumption of animal-sourced protein is higher, with the plant- and animal-based foods, for

100 instance, providing 32% and 68% of protein to consumers in the US, respectively (**Wu et al.**,  
 101 **2014**). Moreover, the demand for animal-based sources is increasing with a rising trend in  
 102 meat consumption in developing countries.

103 **Table 1:** Protein content from different food sources

Source	Animal-based	Protein content (g / 100 g)
<b>Meat</b>	Chicken breast (grilled, without skin)	32
	Pork chop (lean, grilled)	29-32
	Beef steak (lean, grilled)	39-34
	Lamb chop (lean, grilled)	29-31
<b>Fish</b>	Tuna (canned in brine)	25
	Salmon (grilled)	25
	Cod (baked)	24
	Mackerel (grilled)	20
<b>Seafood</b>	Crab (canned in brine)	18
	Mussels (cooked)	18
	Prawns (cooked)	15
<b>Eggs</b>	Chicken egg (whole, boiled)	14
<b>Dairy</b>	Whole, semi-skimmed and skimmed milk	3-5
	Cheddar cheese	25
	Reduced-fat cheddar	28
	Cottage cheese	9
	Plain Greek-style yogurt	6
	Plain low-fat yogurt	5
	<b>Plant-based</b>	<b>Protein content (g / 100 g)</b>
<b>Pulses</b>	Red lentils (boiled)	22 (7)
	Lentil flour (air-classified fraction)	50-57
	Chickpeas (canned)	25 (7)
	Lentils (boiled)	25 (9)
	Chickpea hummus	11
<b>Beans</b>	Soybeans (green, cooked)	22 (12)
	Soybean (air-classified fraction)	55-60
	Tofu (steamed)	8
	Kidney beans (canned)	23 (7)
	Baked beans	5
	Lima beans	11
	Kidney, Black, Navy, Cannellini beans	12.5
<b>Nuts</b>	Almonds	21.1
	Walnuts	14.7
	Hazelnuts	14.1
	Peanut (butter)	25 (26)
	Sunflower seeds	17
	Almond milk	0.4
<b>Grains</b>	Wheat, bread	10-15
	Rice, long grain	7-8
	Corn	9-10
	Oat	15-17

104 \*Sources: British Nutrition Foundation (BNF, 2021), Johns Hopkins Medicine (Medicine, 2019), and USDA (USDA, 2019)

105 Since 1961, the average global per capita meat consumption has increased by 20 kg  
106 (Figure 1). On a worldwide basis, the average annual meat consumption, 40 kg per capita in  
107 2013, is estimated to increase to 51.5 kg by 2050. Increased meat production will require  
108 largescale increase in animal farming. Moreover, one-third of the global cereal production is  
109 used to feed livestock (FAO, 2017) and it is expected to stagnate over the next few decades  
110 (Herrero, 2013). Thus, increasing meat production to such massive scales will be  
111 challenging and costly to the environment.

### 112 Climate risks associated with increased livestock production

113 FAO's Tackling Climate Change Through Livestock (2013) report estimated an  
114 annual emission of 7.1 billion tons CO<sub>2</sub>-eq from livestock (Gerber et al., 2013), 20% of the  
115 35.3 billion tons of global greenhouse gas (GHG) emissions (Ritchie and Roser, 2020). A  
116 comparison of GHG emissions from different food sources (Figure 2A) illustrates that  
117 emissions from animal-based sources are more than 10 times higher than plant-based  
118 sources. Similarly, land use per unit of protein production by animal sources is more than  
119 100 times higher than cereals (Figure 2B). Apart from GHG emissions and habit losses, an  
120 increased shift towards animal-based foods can also result in the introduction of pathogens  
121 into food systems (Figure 2C). Encroachment by humans into natural habitats through  
122 animal farming, hunting, and urbanisation lead to habitat fragmentation which can cause the  
123 emergence of infectious diseases (Newbold et al., 2015). Examples include epidemic  
124 causing coronavirus diseases, all of which originated from bats and market civets (Cui et al.,  
125 2019; Goldstein et al., 2018). The potential for spill over of these bat-borne coronaviruses  
126 to humans through animal foods has been evidenced in literature (Cui et al., 2019).  
127 Ecosystem imbalance due to deforestation and biodiversity loss is another outcome of  
128 intensive animal farming, which intensifies human-wildlife interactions. The risk of  
129 biodiversity loss could be lowered by reducing livestock farming.

### 130 Antibiotic resistance associated with livestock production

131 Another issue with overreliance on animal products is the extensive use of antibiotics  
132 in animal production. High intensity livestock-farming often comes with use of antibiotics to  
133 reduce growth or kill off bacterial pathogens. Animal farming therefore encourages formation  
134 of resistant bacterial strains, which can present downstream problems regarding health and  
135 costs for the livestock, humans, or the environment (Van Boeckel et al., 2015). Although  
136 antibiotics are also used in plant agriculture to prevent bacterial diseases in high-value crops  
137 such as fire blight or bacterial spot (Haynes et al., 2020), the use appears limited. Available

138 data suggests that the USA is the largest employer of antibiotics in plant agriculture, though  
139 the magnitude is much lower compared to livestock agriculture (0.1% vs 75% of antibiotic  
140 use in the USA) (FAO, 2019). Plant-derived protein sources may therefore lower  
141 antimicrobial pollution, provided that antimicrobial use is regulated and monitored. These  
142 data encourage to reduce livestock farming and look for alternative protein sources for a  
143 sustainable future.

#### 144 **Progress and limitations into research for sustainable sources**

145 Scientists are working on multiple fronts to tackle the issue of sustainable protein  
146 production and considerable progress has been made in terms of low emission livestock  
147 production in rangeland farming, alternative protein sources such as insects, algae, and  
148 aquaculture. However, concerns exist about the sustainability of these sources.

149 The use of sustainable practices in rangeland farming such as the use of rotational  
150 grazing systems to reduce overgrazing and soil degradation can help to reduce  
151 environmental impact including GHG emissions (Baronti et al., 2022). Other strategies  
152 include improving feed efficiency, reducing waste and emissions from manure, and using  
153 renewable energy for farm operations. Recently, some farmers have started experimenting  
154 with adding seaweed to feed as a small amount of dietary seaweed can significantly reduce  
155 methane emissions from livestock (Vijn et al., 2020). However, there are still many  
156 challenges needed to be addressed to make these strategies sustainable for farmers. For  
157 example, **1)** implementing new infrastructure can be expensive particularly for small farms,  
158 **2)** challenges in disseminating knowledge and technical expertise can limit farmers' ability to  
159 adopt these practices, **3)** regulatory frameworks may not support adopting sustainable  
160 practices due to lack of government support or friendly policies, particularly in developing  
161 countries, and **4)** there is still limited research on best practices to reduce GHG emissions  
162 from livestock. Therefore, a collaborative effort between farmers, scientists, and  
163 policymakers will be required to make these practices sustainable and affordable for  
164 farmers.

165 Insects are already established in regional meals, examples include, mopane  
166 caterpillars, palm weevil larvae, adult crickets, honeybees, or meal worms, and qualify as a  
167 nutritional alternative to meat. The protein content in insects is similar to meat but shows a  
168 larger variation between species (10 – 35%) (Payne et al., 2016). However, despite their  
169 potential for protein nutrition, there remain certain issues with using insects as food. The  
170 largest variable is reluctance to eat insects in many cultures, though people are willing to try  
171 insect products in unrecognisable forms like powders or supplements in pasta or crisps

172 **(Wilkinson et al., 2018)**. More pioneer work is needed to assess insects' capacity to rival or  
173 replace livestock-protein.

174 Algae encompass different unrelated multi- and single-celled photoautotrophic  
175 organisms, which are considered a potential alternate food source **(Figure 3)**. The  
176 microalgae *Spirulina sp.* and *Chlorella vulgaris* have a higher dry-weight protein content of  
177 up to 58% and 63% **(Becker, 2007; Tokuşoglu and Ünal, 2003)**. *Spirulina* also has 180%  
178 more calcium compared to milk and 670% more protein than tofu **(Capelli and Cysewski,**  
179 **2010)**. Besides being a rich protein source, algae are used as production platforms for food  
180 supplements such as vitamins, pigments of visual or antioxidant value, fatty acids, and  
181 polysaccharides **(García et al., 2017)**. Algae can accumulate significant biomass relatively  
182 fast, function as CO<sub>2</sub> sink, take no farming space for cultivation, and can grow anywhere that  
183 water and nutrients are available, including seawater. Given the many benefits of algae, why  
184 are they not more broadly established as a major food source? A major reason is their taste,  
185 smell, and texture. Many microalgae have a distinct aroma, including earthy,  
186 seafood/seashore, cucumber or smelly summer-pond **(Francezon et al., 2021)**. The  
187 digestibility of microalgae can also vary a lot depending on the presence and composition of  
188 cell walls, with cyanobacteria generally achieving higher scores **(Niccolai et al., 2019)**.  
189 Although digestibility can be improved, it may require costly procedures such as heating,  
190 drying, enzyme treatments, or fermenting. Many microalgae can be genetically altered  
191 relatively easily (with the curious exception of *Spirulina*) **(Jester et al., 2021)**; however, the  
192 growth of genetically modified algae in economically viable open ponds is currently debated  
193 due to ethical concerns **(Henley et al., 2013)**. Another limitation of farming freshwater algae  
194 is an adequate supply of water, for example in Australia, Northern Africa, the Middle East,  
195 and Western China. In summary, despite their many benefits, there remain significant  
196 obstacles to overcome for algae to be considered as viable alternatives to meat.

197 In recent years, aquaculture – a practice of farming fish in controlled environments  
198 such as water ponds or cages, has gained attention as a potential alternative source of  
199 protein. Aquaculture appears more sustainable compared to traditional animal farming due  
200 to less land and water use and lower GHG emissions **(Subasinghe et al., 2009)**. Also, fish  
201 are excellent sources of protein, omega 3, and other essential nutrients. Aquaculture can be  
202 extended to any areas and easily provide the supply of fish and protein to areas where other  
203 sources are scarce.

204 However, there are also challenges associated with aquaculture. These include  
205 issues such as large amount of feed requirement which comes from wild fish populations in  
206 forms of fishmeal or fish oils **(Cao et al., 2015)**. This brings a question about the



207 sustainability of feed used in aquaculture, particularly if it contains non-renewable  
208 ingredients. Other issues such as disease management and environmental impacts in terms  
209 of nutrient pollution and escapees from farms are concerning. In the next sections, we will  
210 discuss how plants can contribute towards global efforts of sustainable protein production.

## 211 **Enhancing plant-based protein sources**

212 To reduce the usage of animal-based foods for protein supply and given the limited  
213 promise of alternative sources such as insects and algae, producing more plant-based  
214 protein may be a viable and sustainable solution to meet the growing demand of protein.  
215 Most plant-based foods come from cereal grains and the protein supply per kg of different  
216 grains is 3-10 times lower than that of meat. For example, chicken breasts (grilled) can  
217 provide 32 g protein per 100 g, whereas wheat as the highest protein cereal provides  
218 between 10-15 g per 100 g dry weight, depending on varieties (**BNF, 2021**). Furthermore,  
219 plant-based protein often has less than the recommended amounts of essential amino acids,  
220 like lysine (**Gorissen et al., 2018**). Historically, it has been difficult to achieve high protein  
221 content in grain because traditional breeding programs focus on achieving high yielding  
222 varieties based on phenotypes, such as plant height, heading date, grain number, grain  
223 weight, and pathogen resistance (**Bedó and Láng, 2015**). Once the selection process  
224 completes and a variety has been produced, from initial screening of 1000s of lines, only  
225 then is analysis of complex traits such as protein content initiated. However, by this stage, a  
226 lot of genetic diversity would have already been lost. This may have led to the notion that  
227 increased grain protein is often associated with decreased grain carbohydrate and yield  
228 (**Tabbitta et al., 2017**). An example of reduced genetic diversity is a major allele that controls  
229 protein content in wheat (*Gpc-B1*, discussed later in detail) was found in a wild emmer and  
230 was not present in the modern cultivars (**Avivi, 1978**). One obstacle faced during traditional  
231 breeding is that in many regions, the window between harvest and sowing is narrow, and  
232 time is required to assess lines in a large breeding program. Nonetheless, emerging high  
233 throughput phenotyping technologies provide new opportunities to screen complex traits  
234 early during the breeding program. For example, **hyperspectral imaging** can rapidly and  
235 non-destructively phenotype grain protein content (GPC) in large-scale breeding trials  
236 (**Caporaso et al., 2018**). To complement these phenotyping approaches, improved genetic  
237 resources including sequenced TILLING populations or diverse germplasm collections and  
238 gene editing technology like CRISPR-Cas9 provide opportunities to discover novel genetic  
239 factors that can enhance our understanding of the biology underpinning complex traits like  
240 protein content.

241 Other plant sources, such as pulses or duckweed, produce considerably higher  
242 protein content than cereals; however, they do not contribute significantly to the overall  
243 human diet in developed countries. For example, the average global pulse consumption (21  
244 g/day) contributes only 3% to the total dietary energy and has remained stagnant since 1985  
245 **(Rawal and Navarro, 2019)**. Therefore, overall cereal production or protein levels in cereals  
246 would need to significantly increase to ensure sustainable protein supply. However, intensive  
247 agricultural farming also puts pressure on the required resources, mainly nitrogen (N) and  
248 water. High crop yields and protein levels are only possible when N uptake is high, hence  
249 commercial crops remove substantial amounts of soil N **(Ewel et al., 2019)**, typically coming  
250 from fertilisers. However, increased N fertiliser application is expensive for farmers, it  
251 contaminates the soil, and raises human health concerns. Higher amounts of nitrates found  
252 in the agricultural lands under N fertilisation can lead to ground water contamination and  
253 cause serious health issues. Some examples include the production of methaemoglobin by  
254 the activity of oral/stomach bacteria converting nitrates into nitrites which leads to inhibition  
255 of haemoglobin activity (blood disorders), or endogenous formation of N-nitroso  
256 compounds (carcinogenic) when nitrites react with acidic stomach **(Ward, 2009)**. Moreover,  
257 N fertilisers contribute to GHG emissions either through the burning of fossil fuels during  
258 manufacture or the denitrification of nitrates into N<sub>2</sub>O by soil bacteria. Human induced N<sub>2</sub>O  
259 emissions into the climate mainly driven by the addition of N fertilisers to croplands have  
260 increased by 30% since the 1980s **(Tian et al., 2020)**. Another major issue with commercial  
261 agriculture to increase crop production is the risk of genetic uniformity within a crop species,  
262 which can drive pathogen susceptibility. For example, the 1970-71 epidemic of Southern  
263 Corn Leaf Blight (SCLB) in several Corn Belt States in the US caused more than 50% loss in  
264 yields in some of the regions, and 20-30% average yield losses on average **(Ullstrup, 1972)**.  
265 Almost 85% of the US corn fields were planting Texas cytoplasmic male sterile (Tcms) corn  
266 in 1970, SCLB only affected Tcms corn. Increasing crop production through modern  
267 agriculture, therefore, remains a challenge.

268 The question then remains: how can we ensure a sustainable supply of protein for  
269 the ever-increasing world population? Here, we propose new approaches for increasing  
270 major plant-based protein sources.

### 271 **Major plant-based protein sources**

272 Major plant-based protein sources in the world behind legumes are cereal grains,  
273 particularly maize, rice, and wheat, which together provide 42% of the total protein in the  
274 developing countries **(Kropff and Morell, 2019)**. Legumes are a healthy source of protein,  
275 have a lower GHG footprint, and improve soil fertility through nitrogen fixation. Some

276 disadvantages include unstable annual yields and low consumption in some of the regions,  
 277 e.g., Caucasus and Central Asia. Fortunately, a recent review is available on the role of  
 278 legumes in addressing protein nutrition challenges (**Semba et al., 2021**). Cereals are a  
 279 major component of our diet and provide more than 50% of the global calorific intake; wheat  
 280 alone accounts for 20% (**FAO, 2022**). Therefore, increasing protein levels in cereals can  
 281 have a significant impact on the overall global protein supply. Among maize, rice, and wheat,  
 282 the protein content of wheat grain is high, and the protein produced comes at cheaper price  
 283 for the consumer (**Table 2**). For the scope of this review, we will focus on wheat as a case  
 284 study and discuss challenges in productivity and increasing protein content. The main  
 285 increase in protein production must come from increased production per unit land area as  
 286 there is limited scope to increase cropping areas.

287 **Table 2.** Price (US\$) per kilo of protein from leading food crops in the world.

Commodity	Price (US\$)/kg <sup>a</sup>	Protein (g)/kg <sup>b</sup>	Protein Price (US\$)/kg <sup>c</sup>
Maize	0.27	104	2.56
Rice	0.34	81	4.24
Wheat	0.31	145	2.16
Oat	0.16	169	0.95
Soybean	0.59	406	1.44

288 <sup>a</sup> Price of commodity from <https://markets.businessinsider.com/commodities> (Accessed: 6 Sep 2022).

289 <sup>b</sup> Protein data is collected from USDA nutrient data laboratory (**USDA, 2019**).

290 <sup>c</sup> Calculated using the equation: (commodity price per kg / protein grams per kg) \* 1000 = protein cost per kg (**Dolson, 2021**).

## 291 **Wheat productivity challenges and the impact of global climate change**

292 Wheat is the 3rd largest cultivated cereal crop, is the most widely grown cereal, and  
 293 has broad impact for more countries than either of maize or rice (**Hanson, 2021**). On  
 294 average, ~677 million tonnes of wheat were produced every year across the world since  
 295 2000 from an average area of ~217 million ha (**FAO, 2022**). Global wheat demand is  
 296 estimated to increase by 60% by 2050. To help overcome challenges related to economic  
 297 and environmental costs of N fertilisers, recent research has focused on improving N-use  
 298 efficiency (NUE) of crops, including wheat.

299 As NUE is a complex trait with many contributing processes, identifying the correct  
 300 trait for improvement is not trivial as key physiological and metabolic processes influencing N  
 301 uptake and utilisation are closely related to yield. This includes the role of the root system,  
 302 nitrate assimilation and its relationship with photosynthesis and post-anthesis  
 303 **remobilisation** and N partitioning, trade-offs between yield and quality aspects (grain N  
 304 content), as well as interactions with capture and utilisation of other nutrients. NUE is defined  
 305 as a product of N-uptake and N-utilisation efficiencies. Traits that influence N-uptake  
 306 efficiency in wheat are root size and morphology, root N transporters, and root interaction

307 with microorganisms (**Foulkes et al., 2009**). Genetics of nitrate transport from soil to plant is  
308 not well understood in wheat. Two gene families of nitrate transporters, *NRT1* and *NRT2*,  
309 have been identified in *Arabidopsis* (**Wang et al., 2012**), whereas 6 and 10 ammonium  
310 transporter genes (*AMTs*) have been identified in *Arabidopsis* and rice, respectively, with  
311 *AMTs* associated with N uptake (**Li et al., 2009; von Wirén et al., 2000**). Some members of  
312 both gene families are expressed in root hairs (**Duan et al., 2016; Gu et al., 2013**), and are  
313 therefore likely to influence N-uptake. Previous studies have indicated the potential to  
314 improve nitrate and ammonium capture from soil by manipulating these N transporters in  
315 wheat (**Liu et al., 2015**); however, no success has been achieved to date in breeding. Key  
316 traits that influence N-utilisation efficiency include the activity of glutamine synthetase that  
317 regulates nitrate assimilation (**Bernard and Habash, 2009**), manipulating photochemistry of  
318 Rubisco which constitutes 30% of the total N in wheat leaves (**Lawlor, 2002**), post-anthesis  
319 **N remobilisation** that is crucial during the grain filling period when soil N falls short, and the  
320 stay green trait that features delayed **senescence** to enhance the photosynthetic capacity of  
321 a plant (**Thomas and Ougham, 2014**). An inverse relationship between grain yield and  
322 protein concentration, makes simultaneous genetic improvement of yield and grain protein a  
323 difficult task. Growers and breeders, therefore, must manage the contradictory objectives of  
324 increased yields and higher protein content. The grain protein deviation (GPD; deviation  
325 from the yield-protein linear regression) (**Bogard et al., 2010**) allows breeders to select for  
326 high protein content without the associated grain yield penalty. Crops that are both high  
327 yielding and high in protein content absorb large quantities of N (**Bogard et al., 2010**).  
328 Because the majority of grain N originates from post-anthesis **remobilisation** (**Gaju et al.,**  
329 **2014**), rather than from post-anthesis uptake, mechanisms to enhance reserve N  
330 accumulation in the canopy and efficiency of **N remobilisation** should also be addressed in  
331 the genetic improvement of GPD.

### 332 **Challenges for phenotyping GPC and NUE in wheat**

333 To achieve the maximum genetic potential of a crop or engineer its genetics to  
334 improve a certain trait, it is critical to establish relationship between genotype and  
335 phenotype. Therefore, high-throughput phenotyping of GPC or N-related traits is as  
336 important as genotyping and all the downstream experiments to build a relationship between  
337 genes and GPC would ultimately rely on accurate phenotyping.

338 The commonly used techniques for N and protein estimation from wheat are the  
339 Kjeldahl (**Mariotti et al., 2008**) and Dumas methods (**Sáez-Plaza et al., 2013**). Both  
340 methods are labour intensive and destructive. Some disadvantages of these methods are  
341 that the Kjeldahl can only measure N bound to free amino acids, nucleic acids, proteins, or

342 ammonium, and not from any other form like nitrates and nitrites, whereas for Dumas  
343 method, high initial cost or small sample size required make it difficult to obtain  
344 representative samples, or incomplete combustion leads to inaccurate measurements  
345 **(Mihaljev et al., 2015)**. Some of the other destructive methods for protein estimation in  
346 cereals are chromatographic and mass spectrometric methods; however, their application is  
347 more focused toward studying the type and quality of protein.

348 Commonly used and emerging non-destructive techniques for phenotyping proteins  
349 involve Nuclear Magnetic Resonance (NMR), Near Infrared Spectroscopy (NIR), and  
350 **Hyperspectral Imaging (HSI)**. NMR is used for studying 3D protein structures and  
351 identifying novel proteins **(Williamson et al., 1995)**, while NIR and HSI are used to  
352 determine protein content. HSI has developed significantly in recent years and is now used  
353 to study protein quality features in grains **(Hu et al., 2021; Ma et al., 2022)**. In grain crops,  
354 the application of HSI has been extended to single grain protein analysis. The potential to  
355 investigate single grains provides an advantage for HSI, because most techniques for  
356 protein estimation in wheat rely on extracting data for batches while ignoring the single grain  
357 variability. However, previous studies have reported that batches are typically bimodal for  
358 protein content and the mean value for the batch does not reflect the true value. For  
359 example, at least two independent studies for protein content on different US wheat classes  
360 have reported variability across **(Delwiche, 1995)** and within batches **(Delwiche, 1998)** on  
361 single grain basis. Variability across single grains has also been reported for other traits in  
362 wheat; for example, falling number **(Caporaso et al., 2017)**, water uptake and protein  
363 content in soft and hard wheats **(Manley et al., 2011)**. These studies show evidence of  
364 single grain heterogeneity in wheat and demonstrate that random selection of all low- or all  
365 high-quality samples can give a misrepresentation of the batch. Therefore, application of HSI  
366 on whole single grains has gained substantial interest from the food industry. As HSI can  
367 phenotype protein content robustly and non-destructively, its use in breeding would help  
368 reduce the time and labour required to phenotype large populations and fast track genetic  
369 analysis to investigate underlying genes. There are, however, certain limitations with the use  
370 of HSI such as the development of a good calibration model, especially when investigating  
371 genetically diverse germplasm. For instance, in case of grain protein, if a population ranges  
372 from 5-20% GPC, the calibration model must include samples with these values so that  
373 future samples can be predicted. Therefore, one standard calibration model based on  
374 hexaploid wheat may not be effective for all kinds of wheat samples. Another challenge with  
375 outdoor HSI calibrations is noise introduced due to inconsistent light. A recent review has  
376 discussed advancements and challenges in the use of HSI for plant phenotyping **(Sarić et**

377 **al., 2022)**. Addressing these challenges would improve the use of technology for industrial  
378 application.

379 Promising remote-sensing technologies for field-based phenotyping for NUE traits  
380 include chlorophyll fluorescence imaging to measure photosynthesis, infrared thermometry  
381 as a proxy for canopy photosynthesis and spectral reflectance vegetation indices, which can  
382 provide accurate estimates of crop biomass and N content. A full review of these phenomics  
383 technologies is beyond the scope of this article. Fortunately, recent reviews of such  
384 phenomics methodologies are available (**Araus et al., 2018; Yang et al., 2020**). Regarding  
385 phenotyping for root system architecture traits, the development of reliable and reproducible  
386 phenotyping technologies will again be critical in plant breeding. Promising techniques range  
387 from small plant approaches (from filter paper and agar to rhizotrons) to field-based  
388 phenotyping (direct approaches, e.g., 'shovelomics' or the soil-core break method or indirect  
389 remote-sensing approaches). Again, recent comprehensive reviews are available  
390 (**Bekkering et al., 2020; Tracy et al., 2020**).

#### 391 **Limitations of wheat protein quality**

392 The quality of wheat proteins is widely debated due to their weak nutritional profile  
393 and potential health concerns. For example, wheat contains less lysine (0.3%) than animal-  
394 based protein (i.e., up to 2.7%) and the high contents of proline and glutamine reduce  
395 digestibility (**Biesiekierski, 2017**). Moreover, wheat gluten proteins can cause allergies,  
396 coeliac diseases, and non-coeliac gluten sensitivity in vulnerable individuals. The prevalence  
397 of gluten-related disorders has reportedly increased in recent years, reportedly affected ~1%  
398 of the population. However, **Shewry & Hey** concluded that it was difficult to establish the  
399 extent to which these disorders had risen because greater reporting could be due to  
400 improved diagnosis and awareness. On the other hand, wheat is not only a major source of  
401 food in many developing countries of Africa and Asia (~70% of total food intake), but it also  
402 provides many essential nutrients including dietary fibres, minerals, and B vitamins.  
403 Therefore, discouraging the use of wheat proteins could in fact have a negative health effect  
404 overall (**Shewry and Hey, 2016**).

405 It is essential to note that breeding efforts have been directed towards improving the  
406 quality of cereal proteins, particularly by elevating lysine content. High lysine lines and  
407 hybrids were developed in maize using the naturally occurring *opaque2* and *floury2* mutants  
408 that show an inverse correlation between lysine and prolamins (reviewed elsewhere (**Yu and**  
409 **Tian, 2018**)). A similar strategy could be adopted in wheat to screen TILLING mutants of  
410 prolamins biosynthetic genes to improve lysine content. A recent study using genomic  
411 prediction has recommended donor genotypes with high lysine content from over 7000

412 winter wheat accessions (**Berkner et al., 2023**), which could further accelerate the breeding  
413 of high lysine wheat. Moreover, gluten variants have been genetically engineered to reduce  
414 antigenicity (**Mitea et al., 2010**). These ongoing breeding efforts highlight the potential to  
415 mitigate the limitations of cereal proteins, including wheat, and the importance of a  
416 multifaceted strategy to ensure sustainable protein production.

### 417 **Genetic progress on GPC regulation in wheat**

418 Early inroads to improve GPC in wheat were made by investigating a collection of  
419 global wheat cultivars characterised by variability of traits in a Soviet Union wheat breeding  
420 program, from a collection of Vavilov Institute of Plant Genetic Resources (VIR)  
421 (**Mitrofanova and Khakimova, 2017**). Earlier studies considered climatic conditions and N  
422 richness in soil as major external factors influencing variation for GPC in wheat. VIR  
423 collections were grown in many parts of the Soviet Union with different soil fertility levels  
424 which led to the understanding that soil fertility (N nutrition) was a major factor involved in  
425 GPC variation. The highest GPC varied between 17-19% in lines grown in south-eastern  
426 regions where the soil N levels were higher (**Ivanov, 1947**). While the earlier studies  
427 indicated environment to be the major factor influencing GPC, later research found genetic  
428 variation in wheat to contribute significantly towards protein levels in grain (**Avivi, 1978**),  
429 suggesting the trait could be improved through breeding; however, progress has been  
430 limited by phenotyping, genetic, and environmental factors described earlier in the review.  
431 Newly developed technologies can accelerate efforts to improve GPC levels in wheat by  
432 adopting a revised breeding strategy, e.g., the introduction of HSI early during the  
433 screening process to select for best yield-protein trade-off, genetic screens of wild  
434 germplasm or the development of TILLING populations to find new genetic variation  
435 (**Figure 4, Key Figure**).

436 The research into high GPC wheat cultivars accelerated between 1960s and 1980s  
437 and a core collection of global wheat genetic resources with high GPC was created. The  
438 collection comprised of wild, weakly domesticated, and cultivated wheat species  
439 (**Mitrofanova and Khakimova, 2017**). Between 1970s and 1980s, intensive work on  
440 improving GPC in wheat by intraspecific hybridisation was carried out in the United States  
441 and Canada and the average GPC in these regions rose by 0.5-3% (**Shewry, 2007**).  
442 However, over the years, it has been seen that GPC is a highly polygenic trait and is strongly  
443 influenced by growing conditions and soil compositions. Until the end of 20th century, the  
444 gene pool available with potential to study for GPC variation in wheat was not fully explored.  
445 In the last two decades, with advancements in molecular genetics and sequencing  
446 technology, the focus has shifted towards identifying loci and alleles contributing to high or

447 low GPC in wheat. The first indication of GPC loci was given in a 1978 study that reported  
448 some accessions of large grain wild emmer (*Triticum turgidum* var. *dicoccoides*) had GPC  
449 genes (**Avivi, 1978**). From these samples, one accession FA-15-3 originating from Israel  
450 was hybridised with a durum variety called Langdon to investigate the chromosomal effect  
451 on GPC (**Joppa and Cantrell, 1990**). These results indicated that the line carrying a pair of  
452 FA-15-3 chromosome 6B markers was highest in GPC. The locus was mapped on  
453 chromosome 6B and named as *QGpc.ndsu-6Bb* (*Gpc-B1*) (**Joppa et al., 1997**). *Gpc-B1* was  
454 introgressed later into bread wheat (**Mesfin et al., 1999**) and physically mapped within a 245  
455 kb region (**Distelfeld et al., 2006**). Apart from this major locus on chromosome group 6,  
456 many other loci have been reported on different wheat chromosomes by independent  
457 quantitative trait loci mapping and genome-wide association studies; however, they were  
458 either not been taken up to investigate their role in the biology of GPC or were  
459 phenotypically unstable.

#### 460 **Biological regulation of GPC in wheat**

461 After the *Gpc-B1* locus was physically mapped on the wheat genome, efforts were  
462 expedited to understand the GPC mechanism in wheat and a gene encoding for a NAC  
463 domain protein was identified (**Uauy et al., 2006b**). The gene was named as *NAM-B1* due to  
464 phylogenetic similarity with the *Arabidopsis* No Apical Meristem (NAM) protein. NAC proteins  
465 belong to a set of transcription factors that are one of the largest plant gene families and are  
466 involved in the regulation of plant development, defence responses, and **senescence**  
467 (**Puranik et al., 2012**). The protein has a conserved N-terminal region (the NAC domain)  
468 with 5 subdomains A-E and a C-terminal domain which is highly variable and has the  
469 transcription activator site (**Uauy et al., 2006b**). The nucleotide comparison of *Gpc-B1* in FA-  
470 15-3 and Langdon wheats revealed that Langdon had a 1 bp insertion at position 933 which  
471 caused a frameshift mutation resulting in 327 amino acid long inactivated polypeptide (**Uauy**  
472 **et al., 2006b**).

473 A comparative study between near isogenic recombinant inbred lines of *Gpc-B1*  
474 active alleles and Langdon wheat revealed that *Gpc-B1* functional alleles increased GPC by  
475 post-anthesis **N remobilisation** from leaves to spikes. The study further reported a high  
476 presence of free amino acids in the flag leaf of the lines carrying *Gpc-B1* active alleles at  
477 anthesis, which suggested *Gpc-B1* affected translocation of N from leaf to grain (source to  
478 sink) and was active much before grain formation (**Kade et al., 2005**). Another study  
479 investigated recombinant substitution lines segregating for *Gpc-B1* functional alleles. The  
480 lines carrying *Gpc-B1* active alleles showed rapid flag leaf chlorophyll degradation after 20-  
481 30 days from anthesis, faster yellowing of main spike peduncles, and reduced moisture



482 content in grain and straw. Consequently, the grain filling period was reduced and the lines  
483 senesced 4-5 days faster, and produced 10-15% higher GPC compared to the wildtype lines  
484 with non-functional *Gpc-B1* alleles (Uauy et al., 2006a).

485 In bread wheat, the *NAM* gene has 4 copies at *Gpc-A1*, *Gpc-D1*, *Gpc-B2*, and *Gpc-*  
486 *D2* loci. *Gpc-B2* and *Gpc-D2* are paralogous copies of *Gpc-B1* on the chromosome group 2  
487 with 91% DNA similarity and 98-100% protein similarity. When the transcript levels of all  
488 *NAM* genes were reduced by RNA interference in a bread wheat cultivar Bobwhite, the  
489 transgenic line with 40-60% reduced transcript levels (between the 4th and 9th day of  
490 anthesis) reached 50% flag leaf chlorophyll degradation 24 days later and main spike  
491 peduncle yellowness 30 days later than the non-transgenic lines. The mature grains of  
492 transgenic plants showed a reduction of 30% in GPC, 36% in Zn, and 38% in Fe; however,  
493 no difference in grain size was detected, relative to non-transgenic lines, which confirmed  
494 that the reduced GPC was a result of low N translocation from the leaf and not a dilution  
495 effect due to larger grains (Uauy et al., 2006b). A more recent study used next generation  
496 sequencing technology to study the transcriptome profile in transgenic lines on the 12th day  
497 of anthesis. The findings of this study indicated that the expression level of several hundred  
498 genes involved in photosynthesis and metabolic processes was changed in the transgenic  
499 lines that led to delayed **senescence** and reduced grain protein, with gene activity changing  
500 greatly before the visual signs of **senescence** (Cantu et al., 2011).

#### 501 **Limitations in the current knowledge and emerging information on the biology of GPC** 502 **in wheat**

503 Many of the findings from different studies, as discussed above, have indicated the  
504 processes of **senescence** and post-anthesis **N remobilisation** to be associated with protein  
505 content in grain. The *Gpc-B1* locus affects the post-anthesis translocation of N from leaf to  
506 grain and as a result plays a pleiotropic role in **senescence** kinetics, grain size and weight,  
507 and GPC. Since the identification of *Gpc-B1* more than 15 years ago, its effect on grain  
508 nutritional components, yield components, physiology, and bread making quality has been  
509 investigated in several field trials worldwide (reviewed elsewhere (Tabbitta et al., 2017)).  
510 Studies have reported varying effects of *Gpc-B1* on yield components with some reporting  
511 increases in yield, while others report no difference. Despite the lack of clear evidence on  
512 the negative effect of *Gpc-B1* on yield, at least three independent studies have reported a  
513 negative correlation between these traits (Brevis and Dubcovsky, 2010; Chee et al., 2001;  
514 Kuhn et al., 2016). Moreover, several studies have reported a negative effect of *Gpc-B1*  
515 functional alleles on grain weight and attributed it to rapid **senescence** and short grain filling  
516 period (Brevis and Dubcovsky, 2010; Joppa et al., 1997; Uauy et al., 2006a). Therefore,

517 a consensus seems to be lacking on the effect of *Gpc-B1* on yield components, which  
518 remains an obstacle for its introgression into the modern wheat cultivars.

519 Recently, new information has emerged which indicates a novel mechanism for  
520 higher protein accumulation in wheat grain without reducing key yield component traits  
521 **(Dixon et al., 2022)**. In this recent study, Dixon *et al.* reported on the role of a  
522 *homeodomain/leucine zipper transcription factor Homeobox domain-2 (HB-2)* in protein  
523 accumulation in wheat grains through changes in the **plant vasculature**. A single nucleotide  
524 mutation in *HB-2* found in the TILLING mutants results in more transcripts of the gene, which  
525 is expressed in cells surrounding xylem and phloem of stems. The mutants produce more  
526 vascular bundles compared to their wildtype siblings, which enhances their hydraulic  
527 conductance. Consequently, more N-based assimilates are translocated to the grain and  
528 GPC increases significantly, with mutants producing grain with ~25% more protein than the  
529 wildtype siblings. Moreover, unlike *Gpc-B1*, the higher protein content of grain from  
530 heterozygous lines was not associated with reduced yield component traits, such as grain  
531 size, and grain weight and number per inflorescence, suggesting moderately higher *HB-2*  
532 expression could help increase GPC without reducing yield **(Dixon et al., 2022)**. This opens  
533 a new research question on the role of **plant vasculature** in **N remobilisation** and the  
534 regulation of grain protein accumulation **(see Outstanding Questions)**. Emerging research  
535 on other crops, such as the discovery of *Teosinte high protein 9 (THP9)* locus from the wild  
536 ancestor of maize which increases seed protein in maize without affecting yield **(Huang et**  
537 **al., 2022)** or the comparisons of storage protein synthesis between monocots and dicots  
538 **(Yang et al., 2023)**, offer approaches for improving GPC in wheat, and vice versa.

### 539 **Concluding remarks and future directions**

540 This study discusses a broader view of the future of plant-based protein, the essence  
541 of increasing protein production to feed the rising population, the impact of increased  
542 demand on the global climate through the enhanced livestock production, and the mitigation  
543 strategies by shifting the focus toward sustainable plant-based protein diets. Although  
544 considerable progress has been made in sustainable rangeland farming, many recent  
545 studies have highlighted the challenges associated with over-reliance on meat production for  
546 protein supply. When we look at the plant-based protein sources, pulses and legumes are  
547 considered because of their high protein content, but the overall global pulse consumption  
548 has not increased since 1965 **(Oecd, 2022)**. On the other hand, cereals could provide major  
549 benefits due to their overall impact as a major part of the global diet, and so any increase in  
550 the wheat protein content can have a massive impact on overall supply. Therefore, we  
551 propose that increasing protein content in cereal grains by using a revised breeding strategy

552 where innovative phenotyping and genetic information can be incorporated early during trait  
553 selection could be a solution.

554 Using genetic and genomic approaches to underpin the molecular control of protein  
555 accumulation in grain and exploiting this knowledge to engineer high quantity and quality of  
556 protein could bring major advances in nutrition. Emerging high-speed testing approaches,  
557 such as **hyperspectral imaging**, offer robust and non-destructive method to phenotype  
558 protein content to help take advantage of the improved genetic resources and knowledge in  
559 wheat. Plenty of information on the genetics of protein accumulation is available, and new  
560 information on the role of **plant vasculature** in the distribution of N-based assimilates to  
561 inflorescences and grain has recently emerged. Such information could be exploited in a  
562 multi-disciplinary approach of combining molecular genetics and high-speed testing to  
563 accelerate breeding for protein rich crops, and ultimately achieve a solution for sustainable  
564 protein supply. Future research could benefit from the following questions:

- 565 **1. Does increasing protein levels in cereals affect the quality of producible**  
566 **protein, and if so, is the trade-off beneficial?** This review discusses an alternative  
567 and more sustainable approach to meet the rising protein challenge through focusing  
568 on boosting protein levels in cereals as they are major contributors of global food  
569 supply. A potential area to explore would be the quality of protein coming from  
570 cereals as an alternative to protein sourced by animal or other plant-based sources.
- 571 **2. Is there potential to improve high-speed testing methods like hyperspectral**  
572 **imaging to screen protein fractions for the determination of protein quality?**  
573 With the emergence of imaging techniques such as hyperspectral imaging into plant  
574 breeding, future research into the suitability of these methods to non-destructively  
575 investigate protein fractions in grain would benefit the grain and protein quality  
576 estimations.
- 577 **3. Do the wheat mutants with altered vasculature showing potential for increased**  
578 **grain protein behave as wild types that may have been treated with low**  
579 **nitrogen?** Nitrogen fertilisers which contribute to environmental and soil pollution are  
580 positively correlated with grain protein content. With the recent discovery on the role  
581 of plant vasculature in grain protein content enhancement without affecting yield,  
582 investigating how the plants with altered vasculature behave in low nitrogen soils  
583 could benefit research seeking to reduce fertiliser use in cropping systems, and  
584 ultimately benefit the environment.

585 **Glossary and Note:**

586 **Plant vasculature**, also known as plant vascular system, is a set of conducting tissues that  
587 transport water and nutrients throughout the plant body. Plants containing vasculature are  
588 called vascular plants or tracheophytes, which comes from Greek word *trachea* (meaning  
589 windpipe) referring to water conducting tissues.

590 **Senescence and nitrogen remobilisation**: Senescence, also known as biological aging, is  
591 directly linked to grain protein accumulation by remobilising nitrogen from vegetative tissues  
592 to grain after fertilisation. Rapid senescence increases grain protein content but can result in  
593 reduced grain carbohydrates and yield.

594 **Hyperspectral imaging** is an emerging high-throughput technique for phenotyping grain  
595 quality traits like protein content. It combines a broad-spectrum camera with near infrared  
596 spectroscopy to extract spectral and spatial information from an object in a three-  
597 dimensional space which can then be used to predict protein content in a single grain using  
598 statistical modelling. The use of hyperspectral imaging early in plant breeding can help  
599 minimise phenotyping time and help capture maximum genetic potential of germplasms.

600 This study included **secondary analysis of existing data** that are publicly available from  
601 FAO at <https://www.fao.org/faostat/en/?#data/QCL>,  
602 <https://www.fao.org/faostat/en/#data/FBS>, and OurWorldinData at  
603 <https://ourworldindata.org/meat-production>. Further information about the terms of use and  
604 licence are available at <https://www.fao.org/contact-us/terms/db-terms-of-use/en/>.

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### 893 **Figure Legends**

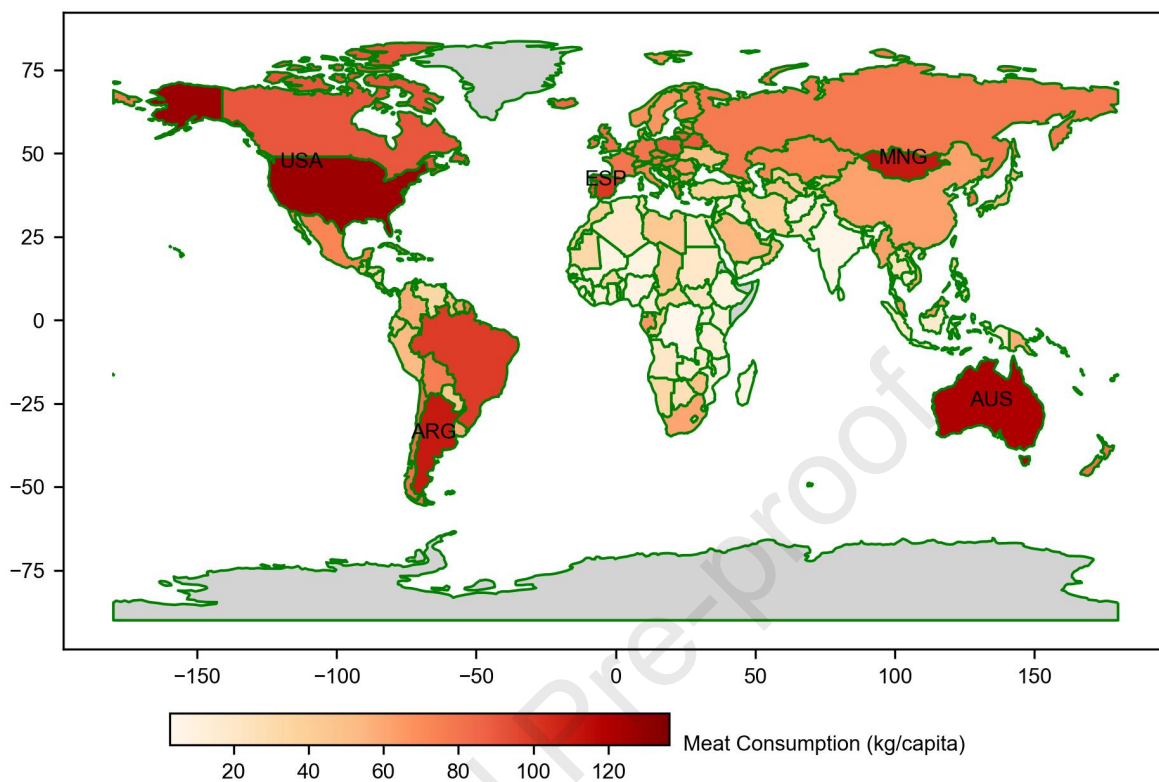
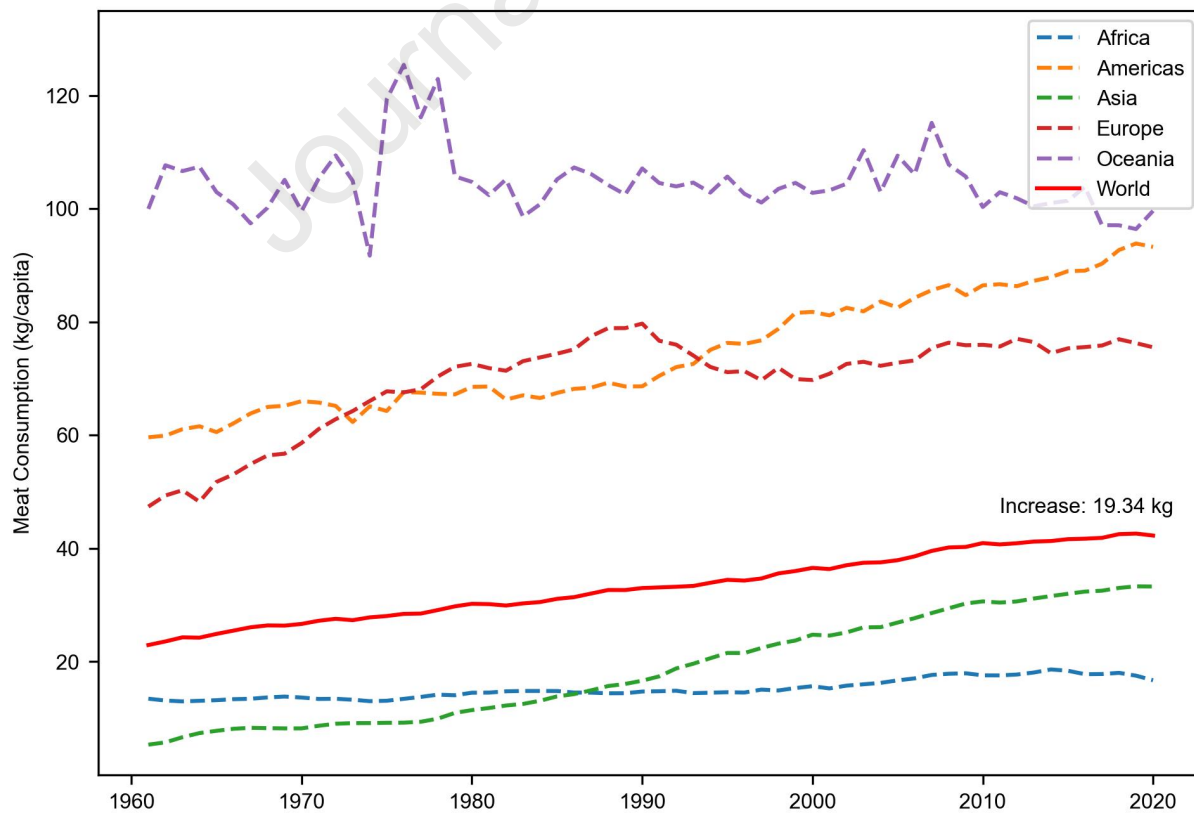
894 **Figure 1. Rising trends in global meat consumption. (A)** Average meat consumption per  
895 capita in 2020. Labels are added for the countries with over 100 kg per capita consumption.  
896 **(B)** Changes in meat consumption trends from 1961 to 2020 across different continents and  
897 globally. Average global consumption has increased by nearly 20 kg. Figure created with  
898 data from Our World in Data **(Ritchie et al., 2017)**.

899 **Figure 2. Climate and pathogenicity risks associated with increased animal farming. (A)**  
900 Greenhouse gas emissions from meat and dairy are highest, whereas emissions from plant-  
901 based foods are more than 10 times less. **(B)** Meat products especially beef and mutton use  
902 at least 100 times larger land area per 100 g of protein compared to cereals. This data further  
903 emphasises how livestock production contributes to global warming and land losses. The  
904 graphs were produced using data from Poore and Nemecek **(Poore and Nemecek, 2018)**  
905 and Our World In Data **(Ritchie and Roser, 2020)**. **(C)** Anthropogenic activities such as  
906 intensive animal farming, wildlife trade, population growth, and urbanisation lead to  
907 imbalances in natural habitats because of biodiversity loss, deforestation, and climate change.  
908 These factors are interconnected and often overlap. As a result, humans are exposed to  
909 wildlife which can cause the spill over of zoonotic pathogens into human populations and lead  
910 to infectious diseases.

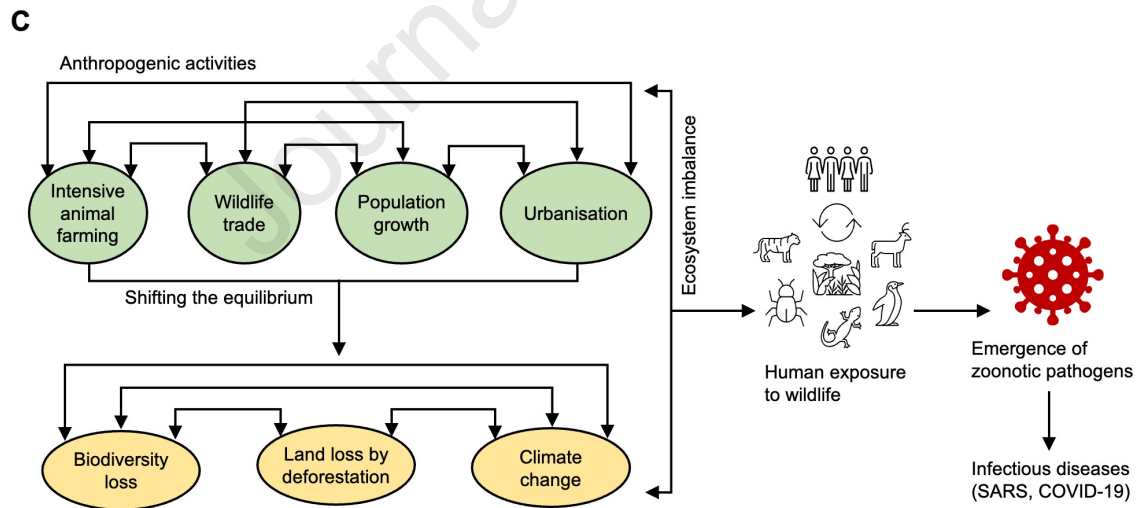
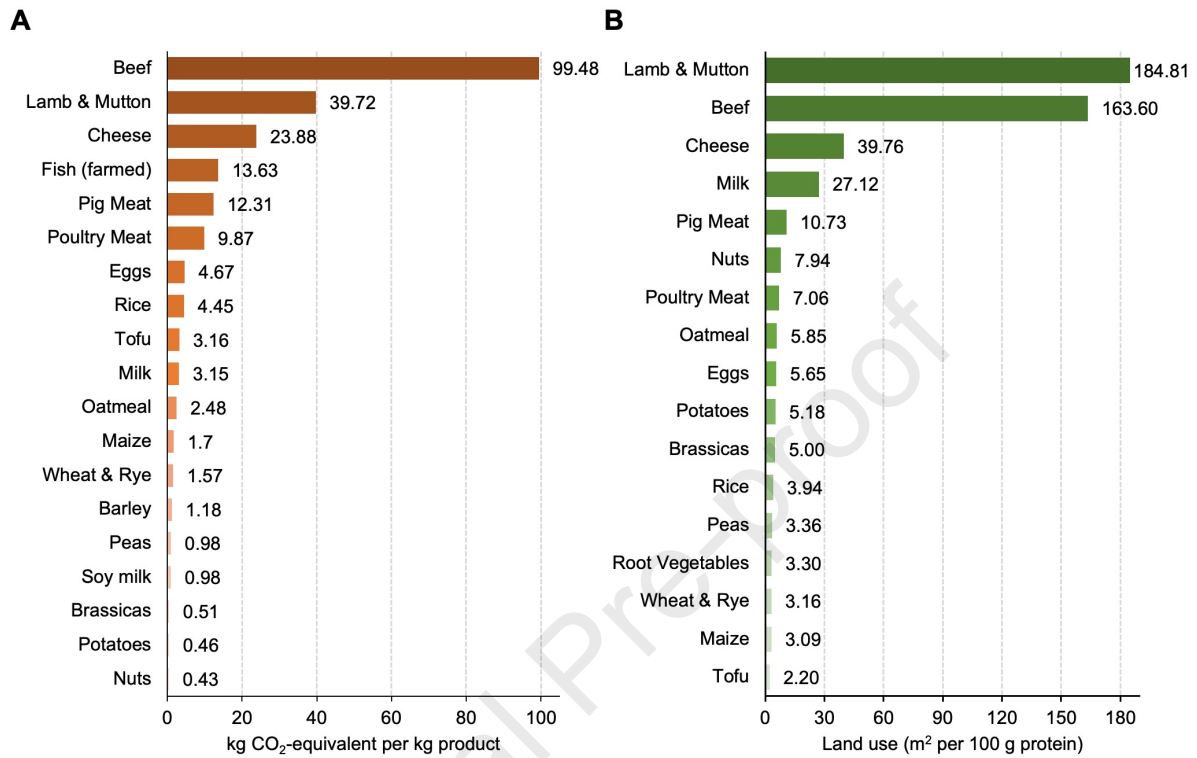
911 **Figure 3. The potential of insects and algae to provide protein in comparison to meat**  
912 **and plants.** The diagram shows advantages and disadvantages of each of these sources in  
913 the context of potential protein supply. + refers to advantage, - refers to disadvantage, ± refers  
914 to intermediary effect. \*Figure created with **BioRender.com**.

915 **Figure 4. A comparison of current breeding pipeline and a proposed new breeding**  
916 **pipeline.** Current breeding pipeline follows the conventional breeding method where

917 thousands of rows are phenotypically screened to select for yield components like grain  
918 yield, height, heading etc. In the later stages, selected few lines are screened for grain and  
919 flour quality characteristics such as GPC, however much of the genetic diversity is lost by  
920 this stage. The new (proposed) breeding pipeline can benefit from high-speed testing  
921 technology like hyperspectral imaging to test GPC in large populations in a rapid and non-  
922 destructive way in real time. On the other hand, advanced genetic tools can rapidly screen  
923 large populations for new variation based on associations between phenotype and  
924 genotype. In this way, quality characteristics like GPC can be targeted much earlier during  
925 the breeding process when the germplasm has maximum genetic potential. \*Figure created  
926 with **BioRender.com**.

**A****B**

Journal Pre-proof







Meat

- + Rich in protein
- + Well established and accepted
- Require lot of space and resources
- Extensive use of antibiotics



Insects

- + Rich in protein
- + Require less space
- ± Can grow fast (with decent-quality food)
- ± Controlled mass farming less established
- Potential for allergens
- Cultural hesitation for consumption



Algae

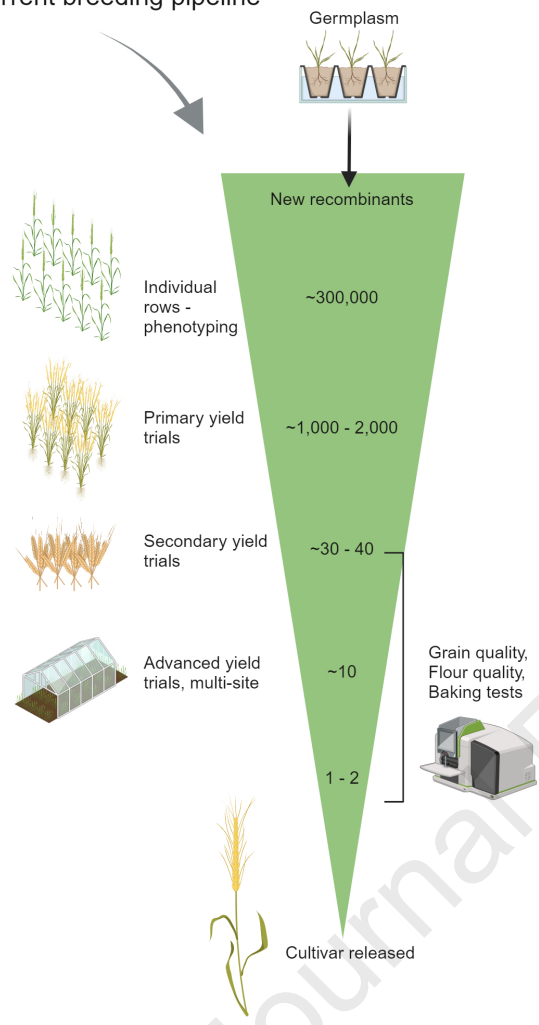
- + Rich in protein
- + Fast and easy growth
- + Little competition for growth locations
- Lack in cheap processing methods
- Often unattractive in taste, smell or looks



Plants

- + Well established and accepted
- ± Little use in antibiotics, potential use in pesticides
- Protein content often low
- ± Potential for genetic modification

Current breeding pipeline



New breeding pipeline

