Received: 24 August 2023 | Accepted: 28 December 2023

DOI: 10.1002/jpln.202300280

RESEARCH ARTICLE

Humalite enhances the growth, grain yield, and protein content of wheat by improving soil nitrogen availability and nutrient uptake

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This article has been edited by Jürgen Augustin.

Funding information
Natural Sciences and Engineering Research Council, Grant/Award Number: ALLRP 566714-21; Mitacs Accelerate, Grant/Award Number: IT-27030; CFI-JELF, Grant/Award Number: 41867; Prairie Mines & Royalty ULC

Abstract

Background: The application of synthetic chemical inputs in current agricultural practices has significantly increased crop production, but their use has caused severe negative consequences on the environment. Humalite is an organic soil amendment that is rich in humic acid and found in large deposits in southern Alberta, Canada. Humic products can enhance nutrient uptake and assimilation in plants by reducing nutrient losses and enhancing bioavailability in the soil.

Aim: Here, we evaluated the effects of different humalite rates in the presence of nitrogen, phosphorus, potassium (NPK) at recommended rates on soil nitrogen availability, wheat growth, grain yield, seed nutritional quality, and nitrogen use efficiency (NUE) under controlled environmental conditions.

Methods: A series of studies were conducted by applying five different rates of humalite (0, 200, 400, 800, and 1600 kg ha^{-1}) with NPK at recommended rates. Soil nitrogen availability and shoot and root growth parameters were recorded at flowering stage. NUE was calculated based on the grain yield at maturity stage.

Results: Plants grown in the presence of humalite augmented root morphological parameters (root length, volume, and surface area), plant biomass (shoot and root), and nutrient uptake (N, P, K, and S) compared to the plants supplied with recommended fertilizer alone. Furthermore, humalite application significantly increased grain yield (14%–19%), seed protein content (23%–30%), and NUE (14%–60%) compared to the fertilizer application alone.

Conclusion: These findings suggest that humalite can be used as an organic soil amendment to reduce synthetic fertilizer application and improve plant growth and yield while enhancing fertilizer use efficiency.

KEYWORDS
humic products, nitrogen use efficiency, plant root simulator probes, roots, shoots

1 | INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops produced for human consumption. It provides more than 20% of calories and protein for daily requirements. It plays a crucial role in underpinning the global food security (Tadesse et al., 2019). Wheat production should be increased by 60% from the current level by 2050 to feed the growing population of ≈10 billion people (Yadav et al., 2020). One of the major inventions of the 20th century for mankind was the production of nitrogen (N) fertilizers by the Haber–Bosch process, which saved billions of people from starvation due to a significant increase in crop yields globally (Erisman et al., 2008; Galloway et al., 2008). After World War II, synthetic N fertilizers were introduced in agriculture to increase crop yields to meet the demand of the rapidly growing population (Ahmed et al., 2017). Furthermore, the green revolution during the 1960s intensified the application of synthetic N fertilizers as several dwarf, nutrient-responsive, high-yielding varieties were developed to boost production. Since then, applying synthetic chemicals to improve plant growth and yield has become common in agriculture. Over the last half-century, crop yields have doubled, but this increase in yield has been achieved with a sevenfold increase in the application of synthetic N fertilizers. This large increase in fertilizer inputs suggests that nitrogen use efficiency (NUE) has declined sharply over time (Han et al., 2016). Excessive application of synthetic fertilizers and pesticides has also led to increasing soil acidification, greenhouse gas emissions, soil salinization, loss of biodiversity, reduced soil fertility, and environmental pollution of both belowground and surface water, thus threatening global food security (Chang et al., 2018; Mu et al., 2021, Sandström et al., 2023; Sharma et al., 2019). Therefore, it is critical to enhance the nutrient use efficiency of plants to maximize production with reduced levels of fertilizer inputs.

Globally, urea is the dominant source of N in agriculture due to its high N content and low cost (Ampong et al., 2022). In order for plants to utilize the N from urea, it is first hydrolyzed into ammonium which is subsequently converted into nitrate ions and subsequently to various nitrogen gases through nitrification and denitrification processes, respectively (Shen, Lin, et al., 2020). Plants can uptake both ammonium and nitrate. However, rapid conversion of ammonium to nitrate reduces NUE as nitrate anions are highly prone to leaching (Barth et al., 2020). Indiscriminate use of chemical fertilizers coupled with nitrate leaching makes the agriculture industry the main source of nutrient pollution and results in serious negative environmental consequences (Ahmed et al., 2017). Reducing soil N application and maintaining crop yield is important for both growers and the environment. Therefore, in the current scenario of global climate change, devising better nutrient management strategies are critical to sustain agriculture production and lower the application rates of synthetic chemical fertilizers, which will reduce the negative impacts on the environment. Thus, there is a pressing need to develop and adopt sustainable and environmentally friendly technologies. One cost-effective and sustainable approach to minimize negative effects on the environment and increase yield is to substitute a portion of synthetic chemical inputs with compounds from natural organic resources.

Humic-based products (HPs) are the most stable component of soil organic matter derived from the biological and chemical transformation of dead biota (Canellas et al., 2015; Nardi et al., 2017). HPs are excellent organic soil amendments known to enhance plant growth and crop yield in several different plant species, including maize (*Zea mays*), wheat (*T. aestivum*), rice (*Oryza sativa*), millet (*Setaria italica*), soybean (*Glycine max*), and canola (*Brassica napus*) (Arslan et al., 2021; Canellas et al., 2019; García, Santos, et al., 2016b; Jannin et al., 2012; Malik et al., 2023; Nunes et al., 2019; Shen, Guo, Wang, Yuan, Dong, et al., 2020; Shen, Guo, Wang, Yuan, Wen, et al., 2020). HPs can improve plant growth through direct and indirect effects. The direct effects of HPs on plant growth and development are associated with different bioactive components such as organic functional groups, amino acids, and phytohormone-like compounds that are enclosed in macromolecular structure. These bioactive compounds activate the signal transduction pathways, reprogramming the expression of a large subset of genes involved in plant growth and development processes such as nutrient uptake and assimilation, photosynthesis, respiration, and primary and secondary metabolism (Canellas & Olivares, 2014; Jannin et al., 2012; Nardi et al., 2017, 2018; Rathor et al., 2023; Zanin et al., 2018). Indirect effects involve the soil environment where HPs improve soil physiochemical and biological properties such as soil structure, texture, water retention capacity, soil pH, enhanced nutrient availability due to the ability of HPs to form complexes with ions that prevent leaching losses, and alterations in microbial abundance and biodiversity (Dawood et al., 2019; García, de Souza, et al., 2016a; García-Mina et al., 2004; Gerke, 2021; Lumactud et al., 2022). These improvements in soil physicochemical and biological properties contribute to a healthy soil environment leading to improved plant performance and increased yield, as observed in HP-supplied plants. Tavares et al. (2019) found that when rice plants were treated with humic acid (HA) obtained from vermicompost, the treated plants showed a significant increase in nitrate and ammonium uptake. A meta-analysis of existing data from 81 studies estimated an ≈22% increase of shoot and root biomass in response to HPs treatment (Rose et al., 2014). Additionally, yield increased by 20% when maize plants grown in poor fertile soils were treated with HPs (Canellas et al., 2013).

The bioactivity of HPs depends on several factors such as origin, environmental conditions, extraction method, and molecular structure making it crucial to find the optimal concentration of HPs application. Excess concentrations of HPs exert toxic effects and negatively affect the growth of plants (Aguiar et al., 2013; Pizzeghello et al., 2020; Rose et al., 2014; Scaglia et al., 2016). The majority of previous studies have been performed using the extraction of HA and fulvic acid from related resources. There have been questions on the bioactivity of these extracted fractions of HPs and one of the key concerns has been that alkali extraction can alter the native structure of HPs (Lehmann & Kleber, 2015). Furthermore, the extraction process is time-consuming and requires standardized procedures and specialized equipment not only for small-scale lab experiments but also commercial industries

(Malcolm & MacCarthy, 1986). We hypothesized that the application of unprocessed humalite will be an effective approach to increase wheat growth and grain yield by enhancing nutrient availability and utilization efficiency of applied fertilizers. This study evaluated the effects of different humalite rates in the presence of nitrogen, phosphorus, potassium (NPK) at recommended rates on soil nitrogen availability, wheat growth, grain yield, seed nutritional quality, and NUE under controlled environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Experiment 1

2.1.1 | Plant growth conditions and humalite treatments

All the experiments reported in this study were conducted under greenhouse conditions at the University of Alberta, maintained at 23°C with a 16-h light/8-h dark cycle and light intensity of 500 μmol photons m^{-2} s^{-1}. The soil used for all experiments was collected from a silage corn stubble field at the University of Alberta Edmonton research farm. The soil was sieved through an 11 mm mesh and uniformly mixed with sand (Target Products Ltd.) at a ratio of 1:2 (v/v). The planting mix was filled into 6.52-L plastic pots, maintaining 7 kg of soil mix pot^{-1}. In all pots, soils were packed to a bulk density of 1.5 g cm^{-3}. The soil mixture was analyzed for macro- and micronutrients at Elements Laboratory. Humalite (WestMet Ag, AB, Canada) was used as the HA-based soil amendment (Table S1). Five different humalite rates 0, 200, 400, 800, and 1600 kg ha^{-1} were tested in this study. All treatments, except the control (soil and sand mix), received NPK fertilizers based on the recommended application rate (N—129, P—32.5, and K—19 kg ha^{-1}) for yield levels of 3.4 t ha^{-1} in the black soil zone as recommended by Elements soil testing lab. The fertilizers and humalite rates for individual pots were calculated based on the soil bulk density maintained at 1.5 g cm^{-3} in all the pots (Thilakarathna & Hernandez-Ramirez, 2021). Humalite was mixed with NPK fertilizer and applied to the top 5 cm of soil. Wheat seeds (var. AAC Brandon 19) were surface sterilized using 3% (v/v) sodium hypochlorite for 2 min, followed by six rinses with sterile distilled water. Three to four seeds were directly placed in the soil and extra seedlings were thinned out, leaving one plant per pot after 1 week of emergence. Plants were watered using distilled water every 2–3 days interval to keep the soil near field capacity. Plants were arranged in a completely randomized block design and the experiment was performed with six biological replicates ($n = 6$) in each treatment.

2.1.2 | Evaluation of shoot and root growth

Plants grown under different rates of humalite and control were harvested at 8 weeks of growth (BBCH: 69). Roots were collected from the soil and thoroughly washed under the running water. Roots were separated individually to spread apart and scanned with a high-resolution scanner (Expression 12000 XL, Regent, QC, Canada). Several individual root scans were obtained for each root sample. Total root length, surface area, and volume were measured with WinRhizo software (Regent). The numbers of tillers and spikes were counted visually. The shoots and roots were oven dried at 60°C for 5 days and dry weight was recorded.

2.1.3 | Estimation of chlorophyll content

The chlorophyll (Chl) content of wheat plants grown under different treatments was determined following a protocol described by Lichtenthaler (1987). In brief, the fully developed young flag leaf was harvested, weighed, and then ground in 5 mL of cold methanol using a mortar and pestle. The ground mixture was centrifuged at 10,000 RPM for 10 min at 4°C. The supernatant was transferred to a fresh tube and the pellet was re-extracted using 5 mL of cold methanol. The final volume was adjusted to 10 mL after combining both extracts. The absorbance of the extract was recorded at 652.4 and 665.2 nm using a Synergy H4 hybrid reader (BioTek) and the Chl content was calculated following the equations described by Lichtenthaler (1987):

$$\text{Chlorophyll } a = 16.72 \times A665.2 - 9.16 \times A652.4, \tag{1}$$

$$\text{Chlorophyll } b = 34.0 \times A652.4 - 15.28 \times A665.2, \tag{2}$$

where *A665.2* and *A652.4* are absorbances at 665.2 and 652.4 nm, respectively.

2.1.4 | Evaluation of soil nitrogen availability

Soil nutrient availability was measured at 2, 4, 6, and 8 weeks after planting by installing three pairs (three cations and three anions) of plant root simulator probes (PRS, Western Ag) in individual pots. PRS probes were installed to the partial depth of the growing media (10 cm) for 2 weeks and replaced every 2 weeks until 8 weeks. The PRS probe can assess nutrient supply rates by continuously adsorbing charged ionic species over the burial period (Hofer et al., 2017a, 2017b). The PRS probes were washed thoroughly with a high-pressure spray of Milli-Q water and sent to Western Ag Innovations Inc. in Saskatoon for available nutrient analysis.

2.1.5 | Evaluation of shoot nutrients content

Whole plant shoots that were harvested at 8 weeks of growth by cutting right above the crown were dried in an oven set at 60°C for 5 days and ground into a fine powder using a grinding mill (SPEX SamplePrep). The mineral elements and N contents were analyzed at the Natural Resources Analytical Laboratory in the Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada. The mineral elements were measured using inductively coupled plasma-optical emission spectroscopy by a Thermo iCAP6300 Duo (CB, UK) and N content was determined by dry combustion method using a Thermo FLASH 2000 Organic Elemental Analyzer (BRE, Germany).

2.2 | Experiment 2

2.2.1 | Evaluation of grain yield, protein content, and NUE

In this experiment, wheat plants were grown to the seed maturity stage under the previously described growth conditions in experiment 1. The experiment was repeated two times with eight biological replicates ($n = 8$) in each experiment. The number of tillers and spikes were counted visually before harvest. The seeds were cleaned manually, and the number of seeds and seed weight were recorded after drying at 60°C for 2 days. The total dry biomass of the aboveground tissues was recorded after drying the tissues at 60°C for 5 days. The seed protein content was analyzed using near-infra-red spectroscopy (Bruker). The NUE of plants was calculated with the following equation (Agegnehu et al., 2016; Salvagiotti et al., 2009):

$$\text{NUE} = \frac{\text{Grain yield with N} - \text{Grain yield without N}}{\text{N applied}}. \quad (3)$$

2.3 | Statistical analysis

All the data reported in this study were tested for the normality and homogeneity of residual variance using the Shapiro–Wilk and Levene's test. Data met the assumptions of normal distribution and equal variance. For the yield parameters, the two individual experiments were combined as there was no significant difference between trial 1 and trial 2 ($n = 16$) that was determined by general linear model followed by multiple mean comparisons. Soil N availability data were analyzed using the two-way repeated measures. Analysis of variance with a confidence level of 95%, followed by the Fisher LSD test with an error rate of 5%, were used to perform mean comparisons. Statistical analyses were performed using Minitab 19.0 (Minitab LLC). Percent changes in the growth and yield parameters were calculated against the NPK fertilizers treatment based on the mean values.

3 | RESULTS

3.1 | Shoot and root growth of wheat plants

Plants grown under different humalite rates showed enhanced shoot and root growth compared to the control and NPK fertilizer application alone (Figures 1 and 2). Wheat plants supplied with different rates of humalite showed higher shoot dry weight (54%, 65%, 62%, and 53% increase at 200, 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) compared to the NPK-alone treatment (Figure 1g; Table S2). The plants grown in the presence of humalite had more numbers of spikes (48%, 57%, 43%, and 48% increase at 200, 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$), and the number of tillers (37%, 37%, 33%, and 38% increase at 200, 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) per plant compared to the NPK-alone treatment (Figure 1h,i; Table S2). Similarly, plants grown in the presence of humalite showed an increase in total root length (31% and 37% increase at 400 and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$), surface area (24%, 25%, and 50% increase at 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$), volume (28%, 54%, 35%, and 62% increase at 200, 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) and root dry biomass at 400 kg ha^{-1} of humalite (99% increase; $p < 0.001$) compared to the NPK-alone treatment (Figure 2g–j; Table S3).

3.2 | Soil nitrogen availability, chlorophyll content, and nutrient uptake in wheat plants

A significant interaction between different treatments and 2, 4, 6 and 8 weeks was found for soil available total N ($p < 0.001$). All treatments including NPK-alone showed a significantly higher ($p < 0.001$) soil available total N at 4 weeks compared to 2 weeks of plant growth (Figure 3; Table S4). However, no significant difference ($p > 0.1$) was found in soil N availability among humalite rates and NPK-alone treatments at both 2 and 4 weeks of growth except for 1600 kg ha^{-1}, which showed higher N availability at 4 weeks (21%; $p < 0.01$). Humalite treatments improved soil available total N between 4 and 6 weeks of growth (Figure 3). No significant difference ($p > 0.1$) was observed in the soil available total N in humalite treatments except for 1600 kg ha^{-1} treatment at 6 weeks. A significant reduction in soil available total N was found from 4 to 6 weeks in humalite 1600 kg ha^{-1} treatment and NPK-alone treatment ($p < 0.001$). After 8 weeks of growth, all treatments including NPK-alone treatment showed a significantly lower N availability compared to 4 and 6 weeks ($p < 0.001$; Figure 3). However, at 6 weeks of growth, the probes showed a higher total soil N availability for the plants grown in the presence of humalite (19%, 24%, and 23% at 200, 400, and 800 kg ha^{-1}, respectively; $p < 0.01$) compared to the NPK-alone treatment. Similarly, after 8 weeks of growth, humalite treatments had higher total N availability at 400 kg ha^{-1} (64%; $p < 0.05$) compared to NPK-alone treatment.

The analysis of shoot nutrient content demonstrated that plants grown in the presence of humalite had higher NPK content (62%, 77%, and 63% increase for N; 65%, 80%, and 73% increase for P; and 49%, 53%, and 42% increase for K at 200, 400, and 800 kg ha^{-1} of humalite, respectively; $p < 0.001$) compared to the NPK-alone treatment (Figure 4a–c; Table S5). Furthermore, the amount of sulfur (S) in shoots of plants was higher (50%, 77%, 71%, and 38% increase at 200, 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) compared to the NPK-alone plants (Figure 4d; Table S5). The estimation of leaf Chl showed that plants grown in the presence of humalite had more Chl *a* and *b*, and total Chl (Chl *a* + Chl *b*) at 400 kg ha^{-1} of humalite (38%, 80%, and 44% increase, respectively; $p < 0.01$) as compared to

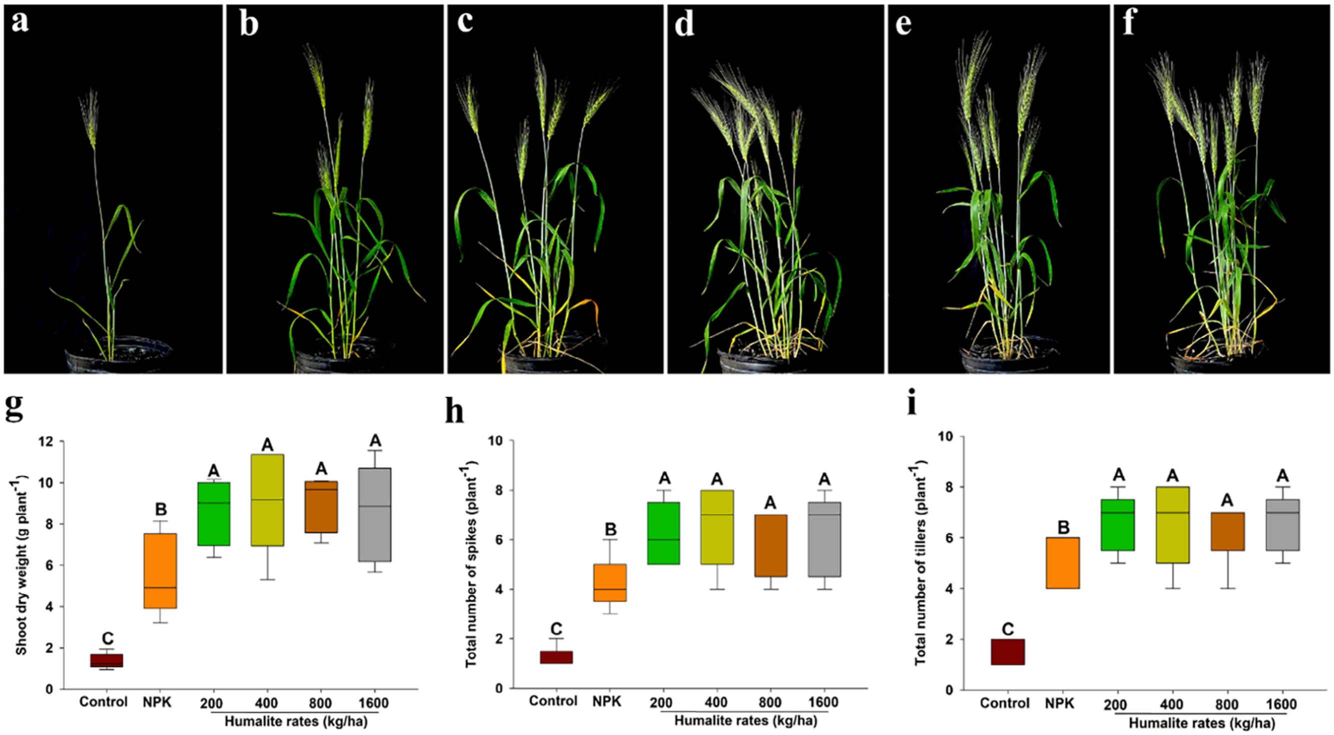

FIGURE 1 Photographs and boxplots of shoot growth parameters showing the median values and variability of wheat plants grown under different rates of humalite and nitrogen, phosphorus, potassium (NPK) at the recommended rate. Plants were photographed 8 weeks after planting. The photographs on the top row from left to right order represent plants from (a) control without NPK and humalite, (b) NPK without humalite, (c) NPK with 200 kg ha^{-1} humalite, (d) NPK with 400 kg ha^{-1} humalite, (e) NPK with 800 kg ha^{-1} humalite, and (f) NPK with 1600 kg ha^{-1} humalite. The graphs in the panels include (g) shoots dry weight, (h) total number of spikes per plant, (i) total number of tillers per plant. Boxplots show the median line and interquartile ranges ($n = 6$). The top line of the box is the third quartile (Q3), whereas the bottom line of the box is the first quartile (Q1). Different letters above the box represent significant differences according to the Fisher LSD test ($p \leq 0.05$).

the NPK-alone treatment (Figure 4e–g; Table S5). The Chl *a*/*b* ratio was reduced in plants grown in the presence of humalite, but the decrease was not significant ($p > 0.2$) (Figure 4h).

3.3 | Plant growth, grain yield, protein content, and NUE of wheat

Wheat plants grown in the presence of humalite also showed a significant increase in plant growth and agronomic parameters compared to the NPK-alone treatment (Figure 5a–f; Table S6). The plants grown in the presence of humalite showed a significant increase in shoot dry biomass (24%, 23%, and 23% increase at 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) compared to the NPK-alone treatment (Figure 5a). The total numbers of spikes (15% and 12% increase) and tillers (13% and 9% increase) were higher at 800 and 1600 kg ha^{-1} of humalite, respectively ($p < 0.001$) compared to the NPK-alone treatment (Figure 5b,c). Similarly, the total numbers of seeds (21% and 20% increase) and total seed weight (17% and 19% increase) were higher than the NPK-alone treatment at 400 and 800 kg ha^{-1} of humalite, respectively ($p < 0.001$) (Figure 5d–e). The total seed protein content (23%, 30%, and 27% increase) of wheat seeds was higher at 400, 800, and 1600 kg ha^{-1} of humalite, respectively ($p < 0.001$) compared to the NPK-alone treatment (Figure 5f). The humalite-treated plants showed an increase in NUE (52%, 62%, and 46% increase at 400, 800, and 1600 kg ha^{-1} of humalite, respectively; $p < 0.001$) compared to the NPK-alone treatment (Figure 6; Table S6).

4 | DISCUSSION

HPs are organic compounds derived from natural resources. These compounds have gained considerable attention in recent years due to their bio-stimulatory effects in improving plant growth and crop yield. Several researchers have observed and documented the plant growth-promoting effect of HPs in different plant species using HPs from a variety of sources (Canellas et al., 2019; Canellas & Olivares, 2014; García, Santos, et al., 2016b; García-Mina et al., 2004; Jannin et al., 2012; Nardi et al., 2018, 2021; Nunes et al., 2019; Scaglia et al., 2016; Shen, Guo, Wang, Yuan, Wen, et al., 2020; Zanin et al., 2018). HPs also play a vital role in improving soil health and plant nutrition and are known to promote several plant growth parameters, such as above- and belowground biomass, leaf Chl, and grain yield (Khan et al., 2018, Muhammad et al., 2015). Photosynthesis is the key metabolic process in plants because it provides energy for growth and development (Baker, 2008). Chl is an important green photosynthesis pigment

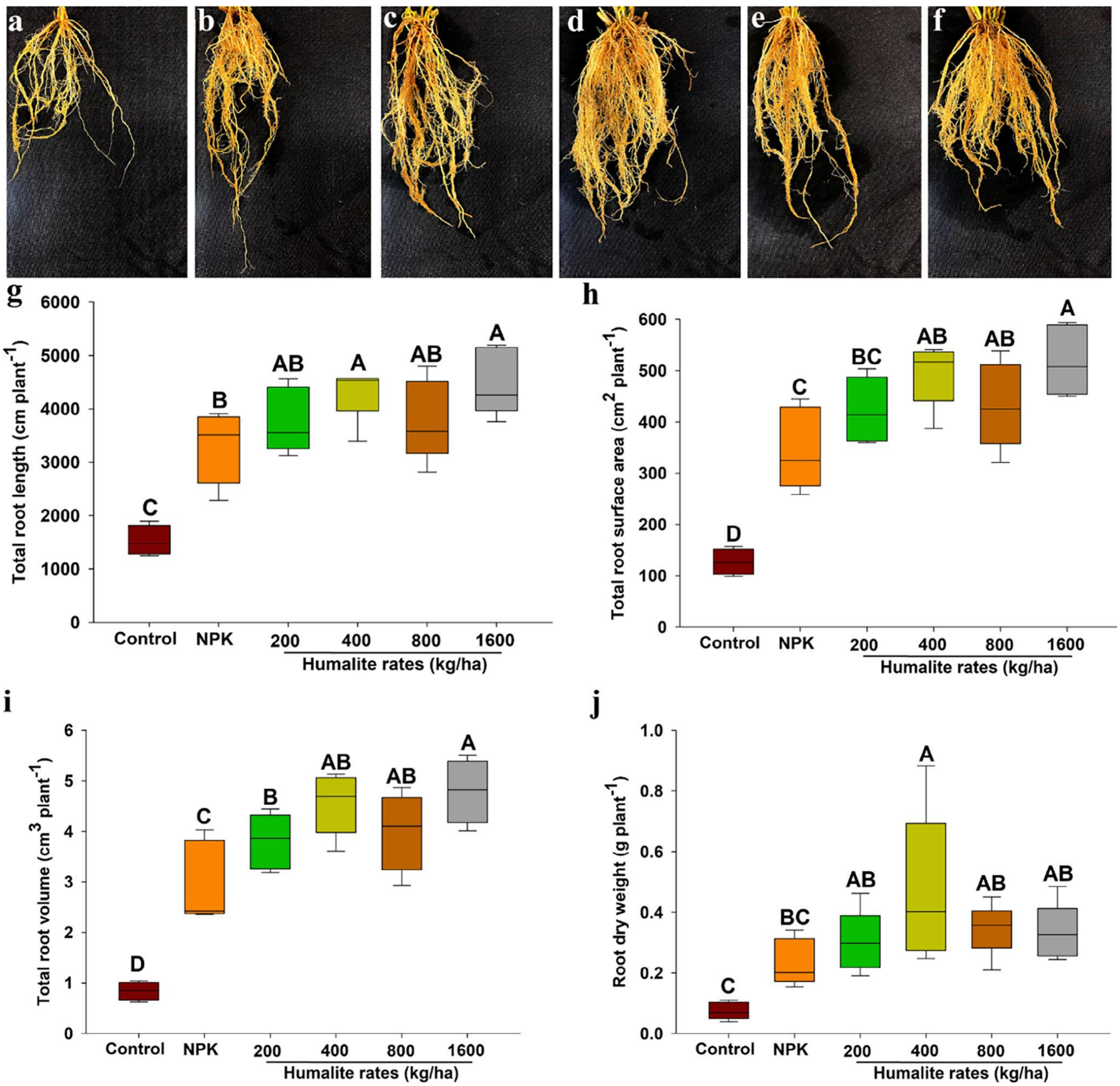

FIGURE 2 Photographs and boxplots of root growth parameters showing the median values and variability of wheat plants grown under different rates of humalite and nitrogen, phosphorus, potassium (NPK) at the recommended rate. Roots were photographed 8 weeks after planting. The photographs on the top row from left to right order represent plants from (a) control without NPK and humalite, (b) NPK without humalite, (c) NPK with 200 kg ha^{-1} humalite, (d) NPK with 400 kg ha^{-1} humalite, (e) NPK with 800 kg ha^{-1} humalite, (f) NPK with 1600 kg ha^{-1} humalite. The graphs in the panels include (g) total root length, (h) total root surface area, (i) total root volume, (j) root dry biomass. Boxplots show the median line and interquartile ranges ($n = 6$). The top line of the box is the third quartile (Q3), whereas the bottom line of the box is the first quartile (Q1). Different letters above the box represent significant differences according to the Fisher LSD test ($p \leq 0.05$).

that regulates the photosynthetic capacity of the leaf (Esteban et al., 2015). In plants, the primary reaction of photosynthesis requires Chl *a* and Chl *b*. The total Chl amount (Chl *a*+*b*) and their ratio (Chl *a*/*b*) directly impact the photosynthesis hence the plant growth and development (Croft et al., 2017; Li et al., 2018). The current study found that humalite application increased shoot and root biomass, leaf Chl, number of tillers, number of spikes, and grain yield compared to the NPK-alone treatments. The increase in number of spikes enhances grain yield in wheat (Muhammad et al., 2015). In the current study, a greater number of spikes per plant was also observed in humalite-treated plants compared to plants that received only NPK (Figure 5b).

One of the classical responses of HPs on plant growth is alterations in root architecture through activation of the auxin signal transduction pathway and enhanced root growth (Canellas et al., 2002; Elmongy et al., 2020; Olaetxea et al., 2018; Rathor et al., 2023; Tahiri et al., 2016; Wang et al., 2017; Zandonadi et al., 2007). Furthermore, the

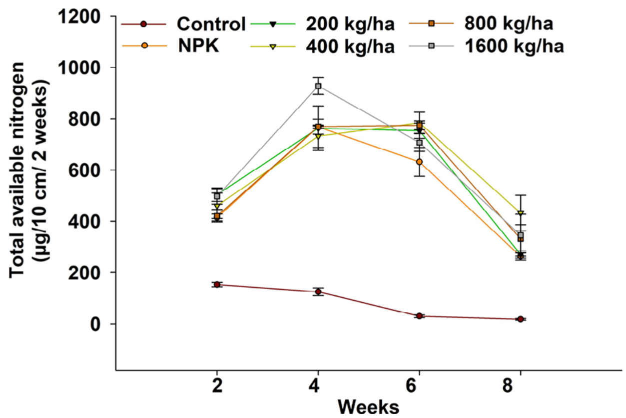

FIGURE 3 Line graph showing the effect of different rates of humalite on total soil available nitrogen estimated using plant root simulator probes at 2-week intervals from the time of planting to 8 weeks. Values correspond to the means ± SE ($n = 6$).

macrostructure of HPs contains indole-3-acetic-acid (IAA) and other similar classes of compounds providing IAA-like activities (Canellas et al., 2002; Muscolo et al., 1998; Russell et al., 2006; Scaglia et al., 2016). Several studies have shown that HPs increase root growth in various plant species (Canellas et al., 2019; Ertani et al., 2019; García, de Souza, et al., 2016a; Jannin et al., 2012; Jindo et al., 2016; Nunes et al., 2019; Olaetxea et al., 2018; Olaetxea et al., 2019; Scaglia et al., 2016). The longer roots can exploit more soil area for efficient water and mineral nutrient absorption (Nibau et al., 2008). In the current study, significant increases in root length, surface area, volume, and dry biomass were recorded in plants grown in the presence of humalite (Figure 2g–j). These increases in root growth traits partly explain the enhanced nutrient uptake and increased plant growth observed in this study.

Nitrogen fertilizers are susceptible to rapid dissipation from soil through surface runoff, leaching, denitrification, and volatilization processes. These losses are a major challenge to growers worldwide as they create an economic cost due to the reduction in NUE of crops and negative impacts on the environment (Fageria & Baligar, 2005). In the current global climate change scenario, reducing N losses and increasing NUE are critical to overcoming the negative consequences of N fertilizers on the environment (Zuo et al., 2018). Several lines of evidence indicate that the combined application of urea with HPs reduces N losses, enhances nutrient uptake, and increases NUE and crop yields (Debska et al., 2002; Dong et al., 2006; Kwiatkowska et al., 2008; Liu, Zhang, et al., 2019; Shen, Lin, et al., 2020). In this study, a significant higher total soil N availability was observed in the rhizosphere with humalite treatments compared to the NPK-alone treatment between 4 and 6 weeks of planting, suggesting higher N availability for longer periods (Figure 3). Furthermore, the higher N content and availability increase the production and accumulation of Chl and enhance the photosynthesis hence the plant biomass (Tsialtas et al., 2012; Wu et al., 2021). The higher total soil N availability and shoot N content found in plants grown in the presence of humalite partly explain the increased leaf Chl content and biomass observed in this study. The higher N content in shoots could be due to the enhanced availability of soil N in the root zone, as evidenced by the data obtained from the PRS probes (Figure 3). HPs are known to enhance the formation of soil macroaggregates, which can improve the water and nutrient retention abilities of the soil, thereby prolonging the effect of fertilizer application (Liu et al., 2020). Furthermore, HPs contain several biochemical functional groups that can form complexes with mineral nutrients thus preventing losses and enhancing availability in soil solution for extended periods (Ampong et al., 2022; Dawood et al., 2019; García et al., 2016a).

HPs have been shown to reduce N losses through inhibition of urease bioactivity by binding to the amino, hydroxy, thiol, and carboxyl functional groups in this enzyme, which reduces the rate of N hydrolysis (Dong et al., 2006; Liu, Wang, et al., 2019; Shen, Lin, et al., 2020). Urease inhibition prevents the rapid accumulation of NH_4^+, which is prone to volatilization, nitrification, and denitrification processes that lead to increased N losses (Guo et al., 2021). Combined application of HPs and urea suppresses the volatilization of ammonia, thus increasing the N availability for extended periods, which enhances N-supply to plants and minimizes the negative impacts on the environment (Ameera et al., 2009; Dong et al., 2006; Guo et al., 2021; Kong et al., 2022; Shen, Lin, et al., 2020). The higher total soil N availability in rhizosphere found in humalite treated plants could be due to reduced N losses from leaching or N_2O emissions as HPs are known to reduce N losses. Kong et al. (2022) found that the application of HA-treated urea reduced the N losses from leaching and N_2O emissions by 25.5% and 23%, respectively, in laboratory experiments performed using field soil. The authors found that HA-treated urea increased the NUE of maize and wheat by 35.3% and 77.5% and yield by 14.2% and 15.5%, respectively. The increases were due to an increase in N availability for extended periods. Furthermore, the combined application of urea and HA increased N availability in the rhizosphere compared to urea alone (Zhang et al., 2019). In this study, a significant higher total soil N availability was observed in the rhizosphere with humalite treatments compared to the NPK-alone treatment between 4 and 6 weeks of planting, suggesting higher N availability for longer periods (Figure 3).

The application of HPs results in diverse environmental and economic benefits. It has been reported that when maize plants were supplied with urea and HA, the shoot biomass, N uptake, and yield increased compared to those plants supplied with urea alone (Zhang et al., 2019). Song et al. (2022) found that when urea was mixed with HA, it improved plant growth and yield of maize by 12.6%–13.9% compared to plants supplied with urea alone. Similar results have also been reported by Liu, Wang, et al. (2019), where the application of HA enhanced the absorption of NPK and increased the yield of maize. In the current study, a significant increase was found in biomass, nutrient content of aerial tissues at the flowering stage, grain yield, and NUE in plants grown in the presence of humalite compared to NPK-alone treatment.

Phosphorus is an essential macronutrient for plant growth and development. It is the key constituent of several biochemical compounds, such as nucleic acids, energy-releasing compounds, and

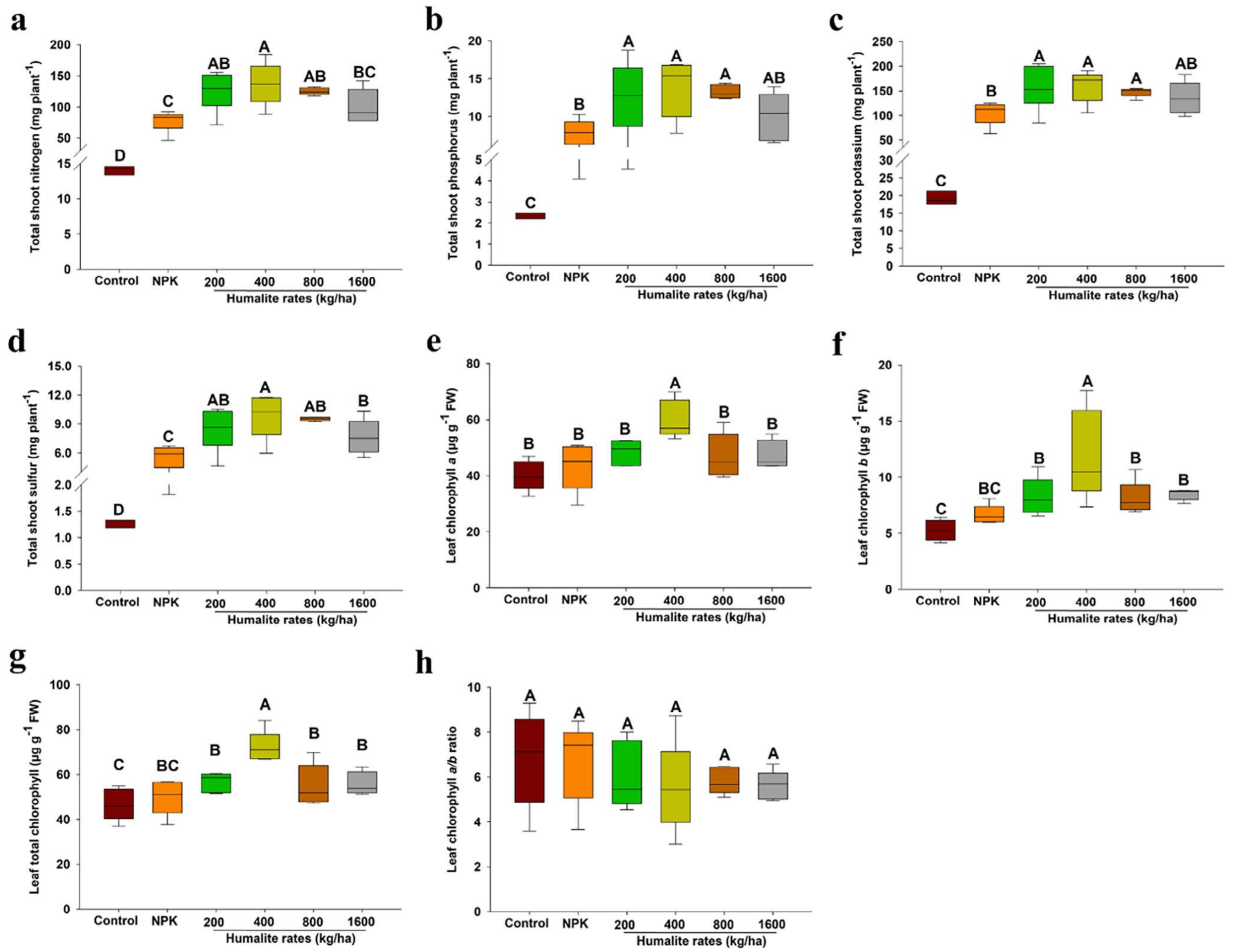

FIGURE 4 Boxplots of shoot mineral elements and chlorophyll content showing the median values and variability of wheat plants grown for 8 weeks under different rates of humalite and nitrogen, phosphorus, potassium (NPK) at the recommended rate. The graphs in the panels include (a) total nitrogen, (b) total phosphorus, (c) total potassium, (d) total sulfur, (e) chlorophyll *a*, (f) chlorophyll *b*, (g) total chlorophyll, (h) chlorophyll *a*/*b* ratio. Boxplots show the median line and interquartile ranges ($n = 6$). The top line of the box is the third quartile (Q3), whereas the bottom line of the box is the first quartile (Q1). Different letters above the box represent significant differences according to the Fisher LSD test ($p \leq 0.05$).

enzymes, thereby playing an important role in determining plant performance and yield (Kratz et al., 2019; Ren et al., 2017). Phosphorus bioavailability in soils for plant uptake is mostly insufficient due to the fixation of P by metal ions into insoluble forms (Cavagnaro et al., 2015; Stutter et al., 2012). HPs can increase P bioavailability by enhancing the transformation of insoluble P to soluble P forms by extrusion of H^+ ions through activation of H^+-ATPase pump and release of organic acids through root exudates (Canellas et al., 2002; Jones & Darrah, 1994; Rathor et al., 2023). Moreover, activation of the H^+-ATPase pump creates the electrochemical gradient required for P mobilization (Raghothama & Karthikeyan, 2005; Rathor et al., 2023). The extrusion of H^+ lowers the soil pH and plant P uptake increases at lower pH as $HPO_3{}^{2-}$ is converted to $H_2PO_4{}^-$, the latter of which is more favorable for plants (Fageria et al., 2017; Yan et al., 2019). Moreover, HPs contain a variety of bioactive functional groups that can attract metal ions and form complexes, thus reducing the P-fixation by preventing the formation of metal-P precipitates (Alvarez et al., 2004; Guardado et al., 2007; Milne et al., 2003; Rosa et al., 2018). Therefore, the increased P uptake observed in this study could be due to increased availability in the soil for plants grown in the presence of humalite (Figure 4b). The results of this study are consistent with previous findings reported by Gao et al. (2023), who also found that the incorporation of HA into P fertilizers increased the biomass, number of spikes, yield, and P uptake of wheat plants compared to P-alone supplied plants.

Sulfur is also an essential nutrient for plants as it plays a key role in the biosynthesis of Chl and protein (Fox et al., 2014). Plant growth and crop yields are significantly reduced under sulfur deficiency. In the current study, a significant increase in shoot S was found in plants grown in the presence of humalite compared to NPK alone (Figure 4d). This increase in S uptake could be due to efficient absorption by roots as humalite application increased root length, surface area, and volume (Figure 2). Yu et al. (2021) showed that sulfur application significantly increased the NUE, protein content, and grain yield of wheat. Akin to the importance of yield increases, increase in grain

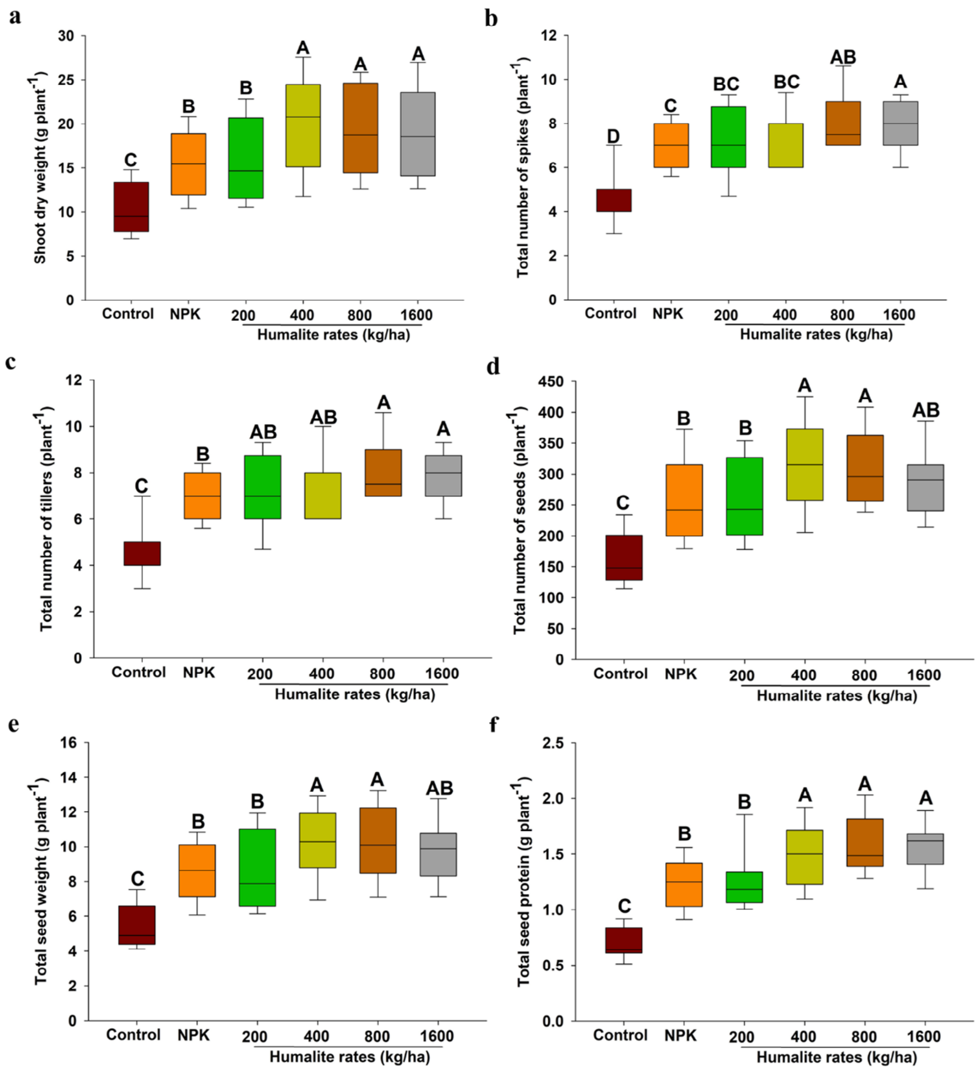

FIGURE 5 Boxplots of shoot growth and yield parameters showing the median values and variability of wheat plants grown under different rates of humalite and nitrogen, phosphorus, potassium (NPK) at the recommended rate. The graphs in the panels include (a) shoot dry weight, (b) total number of spikes per plant, (c) total number of tillers per plant, (d) total number of seeds per plant, (e) total seed weight per plant, and (f) total seed protein content. The experiment was repeated two times with each experiment having eight biological replicates. Boxplots show the median line and interquartile ranges ($n = 16$). The top line of the box is the third quartile (Q3), whereas the bottom line of the box is the first quartile (Q1). Different letters above the box represent significant differences according to the Fisher LSD test ($p \leq 0.05$).

nutritional quality results in economic gains for farmers and a more nutritious food source. In the current study, a significant increase in the protein content of grains was found in plants grown in the presence of humalite compared to NPK-alone application (Figure 5f). The increased protein content could be due to higher N availability and uptake by humalite-treated plants.

The mineral nutrient contribution directly from humalite was negligible (Table S1) and it cannot be argued from these results that mineral nutrients supplied from humalite stimulated the enhanced plant growth observed in this study. Additionally, the results reported in our study follow the same trend as reported in the meta-analysis by Rose et al. (2014) on the effectiveness of HPs in improving plant growth and yield, where an average of 20% increase in plant growth was found in different plant species supplied with different HPs.

5 | CONCLUSIONS

Application of humalite along with the recommended rate of NPK for average yield showed an overall positive effect on different plant growth parameters of wheat under a controlled environment. In this study, we found that humalite application increased wheat growth by improving soil N availability and enhancing nutrient uptake at the flowering stage. However, the data recorded on soil N availability did not take into consideration the soil N that could have accumulated in lower depths, as probes can reach only the top 10 cm of the growing substrate. Further analysis at the maturity stage indicated that humalite application increased NUE, grain yield, and seed protein content compared to the NPK-alone treated plants. In the current study, the application of humalite at 400 and 800 kg ha^{-1} showed a significant

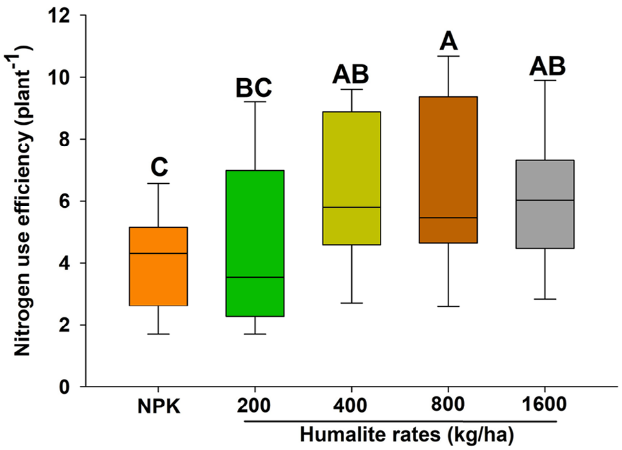

FIGURE 6 Boxplots of nitrogen use efficiency showing the median values and variability of wheat plants grown to seed maturity under different rates of humalite and nitrogen, phosphorus, potassium (NPK) at the recommended rate. The experiment was repeated two times with each experiment having eight biological replicates. Boxplots show the median line and interquartile ranges ($n = 16$). The top line of the box is the third quartile (Q3), whereas the bottom line of the box is the first quartile (Q1). Different letters above the box represent significant differences according to the Fisher LSD test ($p \leq 0.05$).

increase in growth and yield compared to NPK-alone treated plants. These results showed that humalite could be used as an organic soil amendment to enhance plant growth and crop yield with potentially reduced nitrogen rates. In the future, more detailed studies using different nitrogen levels in combination with humalite and investigating the effects of humalite on reducing N loss through volatilization, nitrification, and denitrification processes would benefit the development of humalite into a commercially viable product.

AUTHORS CONTRIBUTION

PR and MT: designing the experiments; PR: writing the original and final draft of the manuscript; PR and VR: performing all experiments and data acquisition; PR and MT: data analysis; PR, MT, VR, LG, and GC: reviewing and editing.

ACKNOWLEDGMENTS

We would like to thank *Kelley Dunfield* and *Shay Missiaen* for their help with the greenhouse trials, *Dr. Bin Shan* for the help with NIR machine and *Nina Mori* for editorial assistance (Department of Agricultural, Food and Nutritional Sciences, University of Alberta). We would like to extend our thanks to WestMet Ag for providing the humalite used in this study. The research was supported by *Natural Sciences and Engineering Research Council* (*NSERC-Alliance grant no. ALLRP 566714-21*), *Mitacs Accelerate* (*IT-27030*), *CFI-JELF* (*No. 41867*), and *Prairie Mines & Royalty ULC*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are in the article itself and in the supplementary material of this article.

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SUPPORTING INFORMATION

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How to cite this article: Rathor, P., Rouleau, V., Gorim, L. Y., Chen, G., & Thilakarathna, M. S. (2024). Humalite enhances the growth, grain yield, and protein content of wheat by improving soil nitrogen availability and nutrient uptake. *Journal of Plant Nutrition and Soil Science*, 1–13. https://doi.org/10.1002/jpln.202300280