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Biodegradation of a Nanocellulose Superabsorbent and Its Effect on the Growth of Spinach (*Spinacea oleracea*)

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ABSTRACT: In this study, spinach (*Spinacea oleracea*) plants were grown in two soils, a clay loam (CL) and a sandy (SD) soil, amended with two types of superabsorbent polymers (SAPs), nanocellulose and commercial, at different levels of soil moisture: 70, 40, and 20%. The effect of the superabsorbent on the soil properties, water management, and plant biomass was measured and compared to that in soils treated with a commercial anionic polyacrylamide-based SAP. Plant biomass is the highest in SD soil amended with a commercial superabsorbent. However, it decreases in the CL soil when a superabsorbent is applied, independent of the SAP type. This effect is magnified when a nanocellulose SAP is used; this is likely attributed to waterlogging stress and the fast biodegradation of this superabsorbent, where approximately 50% of the initial mass remains after 5 days of exposure. The use of a nanocellulose SAP as a water retention agent offers the potential for a much-needed sustainable solution for global agriculture. Future studies are needed to modify the structure of the nanocellulose SAP to inhibit its biodegradation and increase its benefits for agricultural use.

KEYWORDS: *nanocellulose, superabsorbent, plant growth, water productivity, biodegradation*

INTRODUCTION

Water stress is one of the most important limiting factors that affect plant growth and crop productivity, especially during the critical growth-stage of the cultivated crop.[1] The severity and longevity of water stress are influenced by many factors including rainfall amount, intensity, and distribution.[2] One vital parameter helping the agroecosystem to ameliorate the impact of extreme weather events (e.g., drought or intensive rain) is the soil water holding capacity (WHC), which measures the amount of water that a given soil can hold for crop use.[3] Any improvement in WHC provides significant benefits for improving plant growth by reducing the effect of water stress.

Superabsorbent polymers (SAPs) can be used to increase soil WHC.[4] SAPs are hydrophilic polymers capable of absorbing large quantities of water and yet remain stable.[5−8] Their use in the horticultural and agricultural industries has improved soil water availability for plants.[9] As water reservoirs, SAPs have increased water retention in different types of soil, significantly reducing the irrigation water consumption.[10,11]

The effect of various types and amounts of SAPs on plant growth was also evaluated. El-Asmar et al.[12] found that superabsorbent addition to soils prolongs survival time of pine seedlings by 90%. Islam et al.[13] and Satriani et al.[14] demonstrated that the SAP effects on plant yield were significantly magnified when plants were grown under deficit irrigation.

One of the main drawbacks of SAPs is that most are fossil fuel-derived polyacrylamides (PAMs) which exhibit poor environmental degradability. These SAPs release acrylate and acrylamide units when they break down in soil. Acrylamide is known to be carcinogenic, imposing a risk to human health and the environment.[15] These increasing concerns have led to the development of environmentally friendly superabsorbents made from natural polymers, including those made from lignocellulosic agricultural wastes[16] or cellulose derivatives such as carboxymethyl cellulose (CMC)[17,18] and hydroxyethyl cellulose.[19] This is because of the unique properties of cellulose, including renewability, biodegradability, and hydrophilicity.[20,21]

Nanoscale cellulose-based superabsorbents can be prepared using the (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-mediated oxidation method.[22] This oxidation process creates highly porous superabsorbents of high pore areas, exhibiting high swelling properties of up to 200 g of water/g of dry fiber.[23] Despite these characteristics, few studies have evaluated the application of these nanocellulose SAPs for agriculture.[24] Zhang, et al.[25] reported that chemically cross-linked superabsorbents made from nanocellulose fibers (NCFs) can be applied as soilless culture medium for plant growth. Bauli et al.[26] encapsulated the NPK fertilizer in a CMC superabsorbent filled with NCFs. These studies reported cellulose-based SAPs to be effective in slowing the release of fertilizers in water and soil. However, few studies have measured the effect of nanocellulose-based SAPs on plant growth in a controlled

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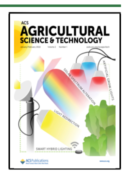

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soil environment not their biodegradability.[27] Limited information relating the impact of these SAPs to plant yield and soil microbial activity is available using different types of soils. Singh et al. recently reviewed a range of materials with their applications in soil and effects on plant growth including cellulosic-based SAPs.[28] A clear understanding of these governing responses is critical for application in agriculture.

Here, we present a greenhouse study in which we quantify the effect of nanocellulose SAPs on spinach (*Spinacea oleracea)* plants grown under different levels of soil moisture (SM) to simulate water-stress conditions. Experiments are conducted using two types of soils in which the total irrigation water required and water productivity (WP) are quantified. Results are compared to spinach grown in the same soils but amended with a commercial PAM-based SAP. It is also our objective to measure the biodegradation of the novel nanocellulose-based SAP and to establish a relationship with the microbial activity.

■ MATERIALS AND METHODS

Materials and Soil Characteristics. Bleached Eucalyptus Kraft (BEK) pulp was provided by Australian Paper, Maryvale, Australia with a chemical composition of cellulose (78.8% ± 0.8), hemicellulose (17.7% ± 0.4), lignin (3.2% ± 0.1), extractives (0.3% ± 0.1), and ash (0.2% ± 0.1).[29] WaterSave BA was purchased from polymer innovations. This is a commercial anionic PAM-based SAP. Sulfate of potash, super phosphate, and urea were purchased from Richgro (Jandakot, Australia). Spinach (*Spinacea oleracea*) seeds were purchased from Mr. Fothergill's (South Windsor, Australia). Seed raising mix was purchased from Yates.

Two soils were used in this study. The first, a clay loam soil, was collected from a vegetable farm located in Werribee, Victoria (37°55′15″S; 144°42′6″E). The second, a sandy soil, was from a vegetable farm converted from pasture located in Cranbourne, Victoria (38°11′6″S; 145°18′50″E). The soils were classified as Red Sodosol and Podosol according to Isbell,[30] respectively. These soils are referred to as clay loam (CL) and sandy (SD) hereafter. Both soils were collected at a depth of 0−20 cm, air-dried, and sieved to <2 mm. Subsamples of 200 g were analyzed for a range of key physicochemical properties (Table 1).[31] Additional properties are given in the Supporting Information (Table S1).

Table 1. Basic Characteristics of the Soils Investigated[a]

parameter	CL (red Sodosol)	SD (Podosol)
pH	8.1	5.7
total carbon (%)	1.3	1.2
total nitrogen (%)	0.12	0.05
C:N	10	25
organic matter (%)	2.2	2.0
effective cation exchange capacity (CEC) ($cmol^+/kg$)	18	2.6
basic texture	CL	SD
WHC (g water/100 g soil)	40	23

[a]A comprehensive soil chemical analysis is provided in the Supporting Information (Table S1).

Superabsorbent Preparation. BEK pulp was used to prepare the nanocellulose SAP following the TEMPO-mediated oxidation protocol previously published to prepare oven-dried superabsorbents,[23] a method adapted from Isogai et al.[32]

Soil Water Holding Capacity. The soil WHC or field capacity of both soils was determined gravimetrically following the method provided by Dane and Topp[3] with values shown in Table 1 as g water/100 g of soil.

Soil Properties. The soil bulk density and porosity were calculated for both soils and with two types of superabsorbents: nanocellulose and commercial superabsorbent. The tests were conducted following a method adapted from Fan et al.[33] Briefly, plastic funnels of 42.5 mm diameter with a filter paper placed at the bottom were weighed (W_0). For each soil type, 20 g of air-dried soil was mixed with 0.5 wt % of either nanocellulose or commercial SAP, added into the plastic funnel, and then weighed (W_1). Treatments were then fully immersed in water for 24 h to allow the SAP to reach saturation. A control treatment for each soil type was also prepared and underwent the same immersion. After 24 h, treatments were raised, and excess water was drained for 3 h. The height of the treatment (from the bottom of the funnel to the top of the soil − to calculate the total soil volume) and mass were recorded (W_2). Treatments were then dried in an oven at 65 °C until constant weight (W_3). Bulk density (g/cm^3) and total porosity (%) were calculated as follows:

$$\text{bulk density} = \frac{(W_3 - W_0)}{V} \times 100 \quad (1)$$

$$\text{total porosity} = \frac{(W_2 - W_3)}{V} \times 100 \quad (2)$$

Greenhouse Spinach (*Spinacea oleracea*) Pot Experiments. A full factorial experimental design was set up in a greenhouse located at Monash University Clayton campus to investigate effects of superabsorbent application and water stress on the growth of spinach. The pot experiments involved two types of superabsorbent, nanocellulose and commercial; three moisture levels, 70, 40, and 20% of soil's WHC; and two soil types, CL and SD soil. Each condition was prepared in replicates of five, giving 90 pots in total.

For each soil type, two treatments of soil amended with the dry superabsorbent were evaluated: nanocellulose at 0.5 wt % (N0.5) and commercial SAP at 0.5 wt % (S0.5). Each treatment was thoroughly mixed and placed in free-draining plastic pots of 14 cm diameter to reach a total of 1.2 and 1.3 kg of soil and superabsorbent for CL and SD soils, respectively. Deionized water was then poured on top of the pots until water drops were observed dripping from the bottom. The pots were then immersed in a tray with deionized water for 24 h to ensure complete saturation of the superabsorbent in water uptake. After 24 h, the pots were raised, and excess water was drained for 3 h. The experiment also included a control treatment in which both soils were not amended with superabsorbent (C0). Controls of both soils were irrigated to saturation to be in line with the other treatments. A basal application of fertilizer was then added to all pots based on soil analysis. The CL soils received N (from urea) at a rate of 100 kg ha^{-1}. For the SD soils, the fertilizer rate was 100 kg ha^{-1} of N, 50 kg ha^{-1} of P (from super phosphate), and 100 kg ha^{-1} of K (from sulfate of potash), respectively. Treatments were then allowed to equilibrate for a further 24 h. Seedlings of spinach, previously grown in trays with seed raising mix, were then transplanted at the two-leaf stage into the pots with soil treatments. For each treatment, three levels of soil moisture (SM) were studied: 70, 40, or 20%.

Once the seedlings were transplanted, treatments were then allowed to reach the desired level of SM. The SM was measured and recorded daily by weighing of the pots and determining the water loss gravimetrically. Once they had reached the required SM, each pot was then irrigated accordingly to maintain this at ±5%. A further fertilizer addition was conducted every 2 weeks at a rate of 20 kg ha^{-1} of N, 20 kg ha^{-1} of P, and 20 kg ha^{-1} of K for both the CL and SD soils. All pots were arranged in a completely randomized block design. A schematic of the experimental setup is displayed in Figure 1.

Conditions in the greenhouse were as follows: for CL soils, temperature 27 °C ± 2 °C day and 19 °C ± 2 °C night and for plants grown in SD soils, temperature 26 °C ± 3 °C day and 16 °C ± 2 °C night. Light levels were maintained with supplemental lighting (12 h day length). Full-spectrum high-intensity discharge lights were used, with the illumination level ramped from 0−30 klux during the 24 h period.

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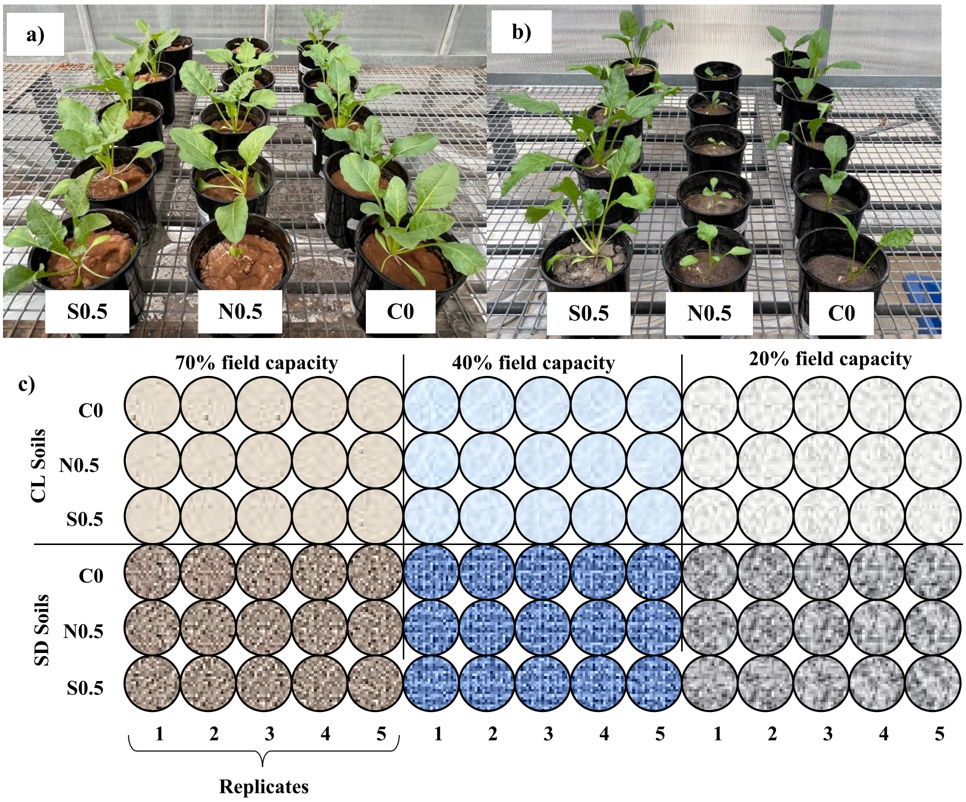

Figure 1. Pictures of the experimental setup at the time of harvesting, (a) spinach treatments in CL soils at 20% of SM and (b) spinach treatments in SD soils at 20% of SM. (c) Schematization of the plant growth experiment. (1) The schematization of the experiment aims to highlight the number of treatments only. All plants were arranged in a completely randomized design. (2) Here, treatments are referred to as control (C0), nanocellulose at 0.5 wt % (N0.5), and commercial superabsorbent at 0.5 wt % (S0.5).

Plant Harvesting and Soil Analysis. Plants in the SD and CL soils were harvested 5 and 6 weeks post-transplanting, respectively. This was performed by cutting the plant just below the cotyledons. Shoots were immediately weighed, and the mass was recorded as shoot-fresh weight. These were then oven-dried for 3 days at 55 °C, and the shoot-dry weight (SDW) was determined. Root biomass could not be reliably recovered because of the nature of the treatments. In CL soils, soil particles highly adhered to the spinach roots, and in SD soils, roots grew through the superabsorbent particles, making them difficult to recover.

Soil subsamples of 10 g were collected from each treatment to measure the amount of nanocellulose superabsorbent remaining at the end of the pot trial. This was performed following the acid digestion method described in the biodegradation tests.

Soil water retention was calculated per treatment as follows:

$$\text{water retention (\%)} = \frac{m_i}{m_o} \times 100 \qquad (3)$$

where m_i refers to the mass of the treatment *i,* and m_o is the mass of the control. Both were fully saturated with water after 24 h.

At the end of the experiment, the total irrigation water required was determined. This value was used to calculate the water use efficiency or water productivity (WP) per treatment by dividing the SDW over the total irrigation water as follows:

$$\text{water productivity (WP)} = \frac{\text{SDW}}{\text{total irrigation water}} \qquad (4)$$

Soil Incubation Studies. Soil incubation studies were performed for both soils and with two types of superabsorbents: nanocellulose and commercial SAP. For each type, 50 g of soil treatments were prepared with 0.5 wt % of application rate of superabsorbent. An additional control treatment was prepared without any superabsorbent for both soils. All treatments received a basal application of fertilizer based on soil analysis. The CL soils received N (from urea) at a rate of 100 kg ha^{-1}, and for the SD soils, the fertilizer rate was 100 kg ha^{-1} of N, 50 kg ha^{-1} of P (from super phosphate), and 100 kg ha^{-1} of K (from sulfate of potash), respectively. Each treatment, thoroughly mixed, was then placed in polypropylene containers of 120 mL, and deionized water was added to reach 70% WHC. Treatments were incubated at 23 °C and 80% relative humidity. Soil moisture was maintained for the entire duration of the experiment by daily weighing of the containers and topping them up with deionized water when necessary. Each treatment was replicated five times.

Gas samples were collected at days 1, 3, 5, 7, 14, 21, 28, and 42 using the static chamber method adapted from van Zwieten et al.[34] Briefly, the containers containing the incubated, treated soils were sealed, and soil gas emissions were allowed to accumulate for exactly 10 min. The accumulated gases were then extracted using a gas tight syringe (SGE Analytical Scientific) and introduced into a pre-evacuated 12 mL Labco exetainer vial through a septum. Collected gases were analyzed for CO_2 on an Agilent Technologies 7890A gas chromatography−thermal conductivity detector (GC-TCD) and a GC-flame ionization detector, fitted with a Gerstel MultiPurpose Sampler autosampler. The detector temperature was 250 °C. Helium was used as the carrier gas at 21 mL/min. The temperature of the column and oven was 60 °C.

Biodegradation Tests. Nanocellulose superabsorbent biodegradation was evaluated in both the CL and SD soils. For each soil, the superabsorbent was added to the soil at 0.5 wt % of application to prepare a total of 10 g of soil treatment. Similar to the soil incubation studies, an additional control treatment was prepared without any superabsorbent for both soils. All treatments received the same basal application of fertilizer specified in the soil incubation studies. Each treatment was thoroughly mixed and placed in polypropylene containers of 25 mL. Deionized water was then added to reach 70% WHC. Treatments were incubated at 23 °C and 80% relative

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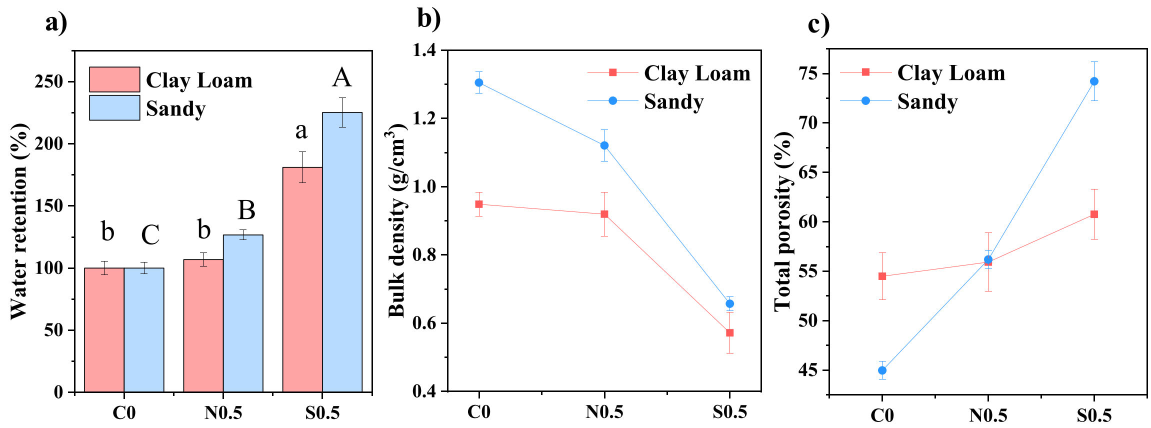

Figure 2. Effect of superabsorbent application on soil properties: (a) soil water retention, (b) bulk density, and (c) soil porosity. Results are reported as mean ± standard deviation (n = 3). Bars with the same letters, lower case (for SD soil) and upper case (for CL soil), were not significantly different at the $p \leq 0.05$ level (Tukey's HSD). (1) Here, treatments are referred to as control (C0), nanocellulose at 0.5 wt % (N0.5), and commercial superabsorbent at 0.5 wt % (S0.5).

humidity. Soil moisture was maintained for the entire duration of the experiment by daily weighing of the treatments and topping them up with deionized water when necessary. Each treatment was conducted in five replicates. Treatments were destructively sampled at days 1, 3, 5, 7, 14, 21, 28, and 42, dried in an oven at 60 °C, and stored frozen at −20 °C until required.

Biodegradation was measured using the acid digestion method adapted from Sluiter et al.[35] to hydrolyze the cellulose and oxidized cellulose in the soil to glucose and glucuronic acid which were then measured by high-performance liquid chromatography (HPLC). In brief, 3 mL of 72% sulfuric acid was added to 5 g of treated soil at room temperature. The mixture was incubated at room temperature for 60 min. During this time, the mixture was occasionally stirred every 5 to 10 min to ensure even acid−soil contact. Dilution to 4% sulfuric acid was then made by adding 83 mL of deionized water followed by autoclaving for 30 min at 121 °C. After cooling to room temperature, the soil was removed by filtration, and the supernatant was retained and neutralized to pH 5 using calcium carbonate. When neutralization was complete, the solids were separated by centrifugation at 4400 rpm for 5 min. The supernatant was collected, filtered, and analyzed by HPLC using a BioRad Amminex HPX-87H column, 0.005 M sulfuric acid as the mobile phase, with a refractive index detector at a temperature of 40 °C, a column temperature of 60 °C, and a flow rate of 0.4 mL/min. Because cellulose is a component already present in every soil, the nanocellulose SAP biodegradation was calculated as follows:

$$\text{biodegradation (\%)}\frac{(M_i - M_c)}{M_t} \times 100 \tag{5}$$

where M_i refers to the mass of cellulose in the treatment at time i, M_c is the mass of cellulose in the control, and M_t means the total mass of cellulose added to the treatment.

Data Analysis. All plant growth, water usage, and biodegradation data were analyzed by two-way analysis of variance (ANOVA) with the factors in the analysis being treatment and SM. Soil respiration and biodegradation data were analyzed by one-way ANOVA with factor in the analysis being treatment and soil type, respectively. Where significant differences were identified, pairwise comparisons were made with Tukey's honestly significant difference (HSD). Analyses were performed using GraphPad Prism 9.0.2.

RESULTS

Effect on Soil Properties: Water Retention, Total Porosity, and Bulk Density. The effect of superabsorbent application on soil properties was measured for CL and SD soils, respectively (Figure 2). For both soils, water retention increases with the addition of SAPs, independent of the superabsorbent type. The water retention in soils treated with a commercial SAP was significantly higher compared to that in both the control and treatments with nanocellulose SAPs. After the 24 h immersion in water, these soils retained approximately 180 and 220% more water than soils without a superabsorbent (control) for the SD and CL soil, respectively. For both types of superabsorbents, the increase in water retention is higher when these are applied to SD soil than for CL soil.

For both soils, bulk density decreases, and soil porosity increases with SAP application. This effect is accentuated with commercial SAPs. For SD soils, bulk density decreases from 1.3 to approximately 0.7 g/cm^3 with the application of a commercial SAP compared to the control. Similarly, the soil porosity in SD soil without the SAP increases from 45 to 75% with the addition of the commercial SAP. Similar effects were observed in both soils treated with a nanocellulose SAP but to a lesser extent. These results of bulk density and porosity agree with previous literature analyzing the application of SAP to soils.[9,36]

Plant Growth Study Summary Analysis. This study determined the effect of treating two types of soil with a nanocellulose superabsorbent on plant growth under water stress. The influence of two factors, treatment (T) and SM, was measured. Significant two-way interactions were found among the different response variables (Table 2). The following sections describe the effect the superabsorbent has on each of the response variables.

Effect of the Superabsorbent on Water Management. Two variables were evaluated to measure the effect of superabsorbent application on water use: total irrigation water required and WP. The total irrigation water required is the amount of water consumed by the plant or lost because of evaporation from the soil during the growing period. WP is defined as the ratio of dry biomass to the total water consumed. The total irrigation water was calculated for each soil type depending on the treatment and SM (Figure 3). The total water required to maintain each of the desired levels of soil SM throughout the growing period is significantly lower in soils amended with the nanocellulose SAP than the control; no significant difference in water required is observed between the control and commercial SAP. Both effects are independent of the soil type or moisture (Figure 3a,c), and both are related to

Table 2. ANOVA Summary of All Response Variables[a]

	T	SM	T × SM
CL soil			
total irrigation water	*	****	**
WP	****	****	****
SDW	****	****	****
SD soil			
total irrigation water	**	****	ns
WPWP	****	***	ns
SDW	****	***	ns

[a]Factors in the analysis were treatment (T) and SM. Both main effects and interactions are indicated. Here, ns = no significant difference, * is $P \leq 0.05$, ** represents $P \leq 0.01$, and **** is $P \leq 0.0001$.

the plant growth. When considered separately from the soil treatments, the total water required in both the CL and SD soils significantly decreases with decreasing levels of SM (Figure 3b,d).

Similarly, the WP was calculated for each treatment and different levels of SM (Figure 4). For the CL soils, data analysis reveals significant two-way interactions between SM and treatment (Table 2). Here, WP significantly decreases with the addition of the superabsorbent, independent of the type of SAP (Figure 4a). The opposite response is observed in SD soils, where WP is the highest in pots with commercial SAP and twice higher than the control, irrespective of the SM (Figure 4c).

Plant Biomass. *CL Soil.* In most cases, the SDW of all treatments decreases with decreasing level of SM (Figure 5). There is a significant two-way interaction between soil treatment and the level of SM (Table 2). The SDW of spinach in treatments with the superabsorbent is significantly lower than the control, at all levels of SM. This effect is more accentuated with the nanocellulose SAP. The SDW in these treatments is approximately five times lower than the control in the pots subjected to 70% of SM. This difference is the greatest of all treatments (Figure 5a,b). When SM is considered, the SDW decreases with decreasing levels of SM, which highlights the response of plant growth under water stress.

SD Soil. No significant interactions are observed between the treatment and SM (Table 2). In general, the SDW decreases with decreasing levels of SM, independent of treatment (Figure 5f). Considering the effect of treatment only, the SDW in pots with the commercial SAP is significantly higher compared to that in the other treatments, irrespective of SM. No significant difference in SDW is observed between the control and those with the nanocellulose SAP (Figure 5e).

Microbial Activity. In general, for both soil types, soil respiration rates (measured as CO_2 emissions) increase with time and application of the nanocellulose SAP (Figure 6). After 40 days, the difference in soil respiration between the control and soils treated with the nanocellulose SAP ranges between 14 and 28 g CO_2/m^2 for SD and CL soils, respectively. These measurements indicate the response of the soil microbial community to the presence of nanocellulose. Similarly, for both soil types, no significant difference is observed in soils amended with a commercial superabsorbent and the controls.

Biodegradation. Biodegradation tests were conducted on the pots treated with a nanocellulose SAP at the end of the plant experiment (Figure 7a). Both soils at all levels of SM were tested. For both soil types, a minimum amount of superabsorbent remains even after over 35 days of exposure. For SD soils, no statistical difference is observed between treatments at any level of SM. However, the nanocellulose SAP in CL soil shows a significant difference when treated at 20% of SM compared to those in 70 and 40%.

The soil incubation study reveals the rate of nanocellulose biodegradation to be dependent on the type of soil (Figure 7b). For both soils, the degradation of nanocellulose shows a similar trend. After an initial exponential increase during the first 7 days of exposure, the SAP degradation rate decreases to reach a plateau. There is a significant difference in nanocellulose biodegradation in both soils at day 7, where 50 and

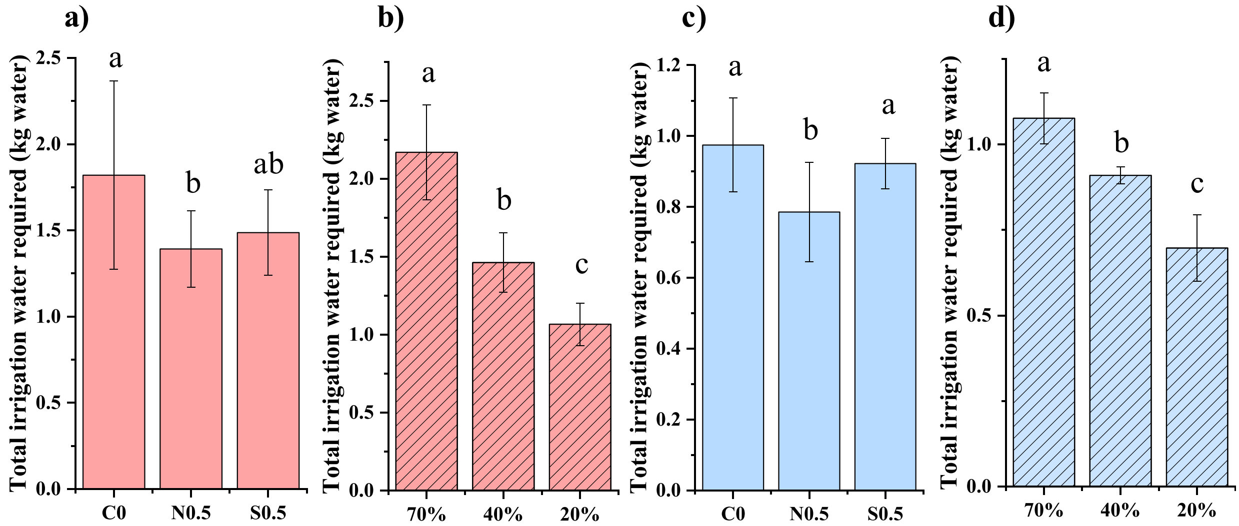

Figure 3. Total water required in the growth of spinach under the different treatments and levels of SM. For CL soil (in red): (a) water loss in treatments irrespective of SM and (b) SM irrespective of treatment. For SD soil (in blue): (c) water loss in treatments irrespective of SM and (d) SM irrespective of treatment. Values are reported as mean ± standard deviation ($n = 5$). Means followed by the same letter were not significantly different at the $p \leq 0.05$ level (Tukey's HSD), see Table 2 for details of ANOVA results. Here, treatments are referred to as control (C0), nanocellulose at 0.5 wt % (N0.5), and commercial superabsorbent at 0.5 wt % (S0.5).

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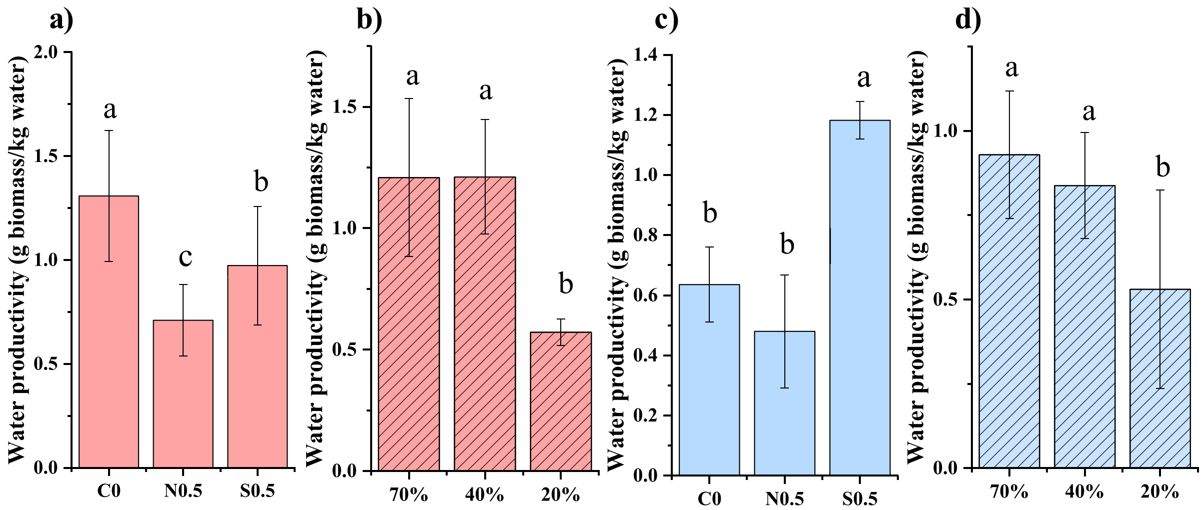

Figure 4. WP of spinach grown in two soil types. For the CL soil (in red): (a) WP in treatments irrespective of SM and (b) SM irrespective of treatment. For the SD soil (in blue): (c) WP in treatments irrespective of SM and (d) WP in SM irrespective of treatment. Values are reported as mean ± standard deviation ($n = 5$). Means followed by the same letter were not significantly different at the $p \leq 0.05$ level (Tukey's HSD), see Table 2 for details of ANOVA results. Here, treatments are referred to as control (C0), nanocellulose at 0.5 wt % (N0.5), and commercial superabsorbent at 0.5 wt % (S0.5).

30% of the initial mass remain in the SD and CL soils, respectively.

■ DISCUSSION

Several studies report that the amendment of SAPs to soils can alleviate the impact of drought on plant growth, especially in arid regions.[11] This is because SAP addition can increase the soil water retention, improving plant growth when water supply is limited.[37] Similarly, studies suggest that the addition of SAPs can improve the soil properties such as bulk density and soil porosity. These reflect the soil's ability to provide structural support, oxygen, and water. Each of these variables indirectly affects plant growth.

In this study, for both soil types, soil porosity increased with the application of superabsorbents. An increase in soil porosity leads to an enhancement in the rate of seedling emergence and seed germination, root growth, and density. Most importantly, it also increases soil aeration, increasing the oxygen availability in the root zone of plants and the soil microbial activity.[9,36,38,39] Similarly, for both soils, the addition of the superabsorbent increases water retention, independent of the type of superabsorbent. This response agrees with previous literature. This is because the SAP granules occupy all the empty pores in the soil and fill them with additional water,[40,41] increasing the soil WHC. However, for both types of superabsorbents, such an increase in water retention is lower in the CL soils. This is due to the swelling mechanism of the SAP, which is driven by the difference in osmotic pressure across the polymer network (difference inside and outside) caused by the movement of the cations in the system.[22] These osmotic effects are diminished with increasing concentrations of salt, resulting in a decrease in swelling.[42] The effective CEC in the CL soil is higher than that for the SD soil, being 18 and 2.6 $cmol^+/Kg$, respectively. In addition, the effective CEC of the CL soil is dominated by the Ca^{2+} ions, known to impact the swelling capacity of superabsorbents.[43]

Analysis of the total water required data revealed that the effect of SM induced a higher response than the effect of treatment in both soils, where the water required significantly decreased with decreasing SM. Differences in water requirement are correlated to the plant growth[44] which can be affected by water deficiency. In particular for spinach, studies have found a decrease in plant biomass when grown under water stress, leading to a decrease in water requirements.[45]

WP varied widely depending on the type of soil. Data analysis revealed that both the treatment and soil type had a significant effect. WP measures the ratio of total biomass to irrigation water consumed. Interestingly, WP decreased in the CL soil with the addition of either SAP. These results contrast with most of the studies that rather suggest superabsorbents to increase WP;[2,13,14,46] this observation is accentuated in treatments at 70% SM. Because WP is related to plant biomass, its decrease is attributed to waterlogging stress which caused a decrease in the growth of spinach. This was noted in the reduction of SDW in treatments with SAPs, at all levels of SM (Figure 5a,b).

Waterlogging occurs when there is an excess of water in the plant's root zone, decreasing oxygen availability and leading to a reduction in plant growth, and in some instances, leading to plant death.[47] The waterlogging effect is important especially in CL soils. Because of the clay content, these soils have a greater WHC than SD soils. In the dispersive soils such as the CL, waterlogging can result in a collapse of the soil structure, blocking pores and leading to poor aeration. The increase in water retention caused by the addition of the superabsorbent to CL soil is likely to have led to a waterlogging stress in spinach plants at the seedling stage. Previous studies have shown that waterlogging stress can appear when high concentrations of SAP to soils are employed.[2,48] This is because superabsorbents can increase soil capillary porosity and decrease the air-filled porosity of soils, resulting in poor aeration.[13] This waterlogging stress led to an overall decrease in plant growth as it is observed in the reduction in SDW in treatments with superabsorbents, both commercial and nanocellulose. Another factor that may have contributed to the reduction in SDW in treatments with nanocellulose SAPs at 70% SM is the fast biodegradation of nanocellulose, occurring during the first week of the study. The high porosity

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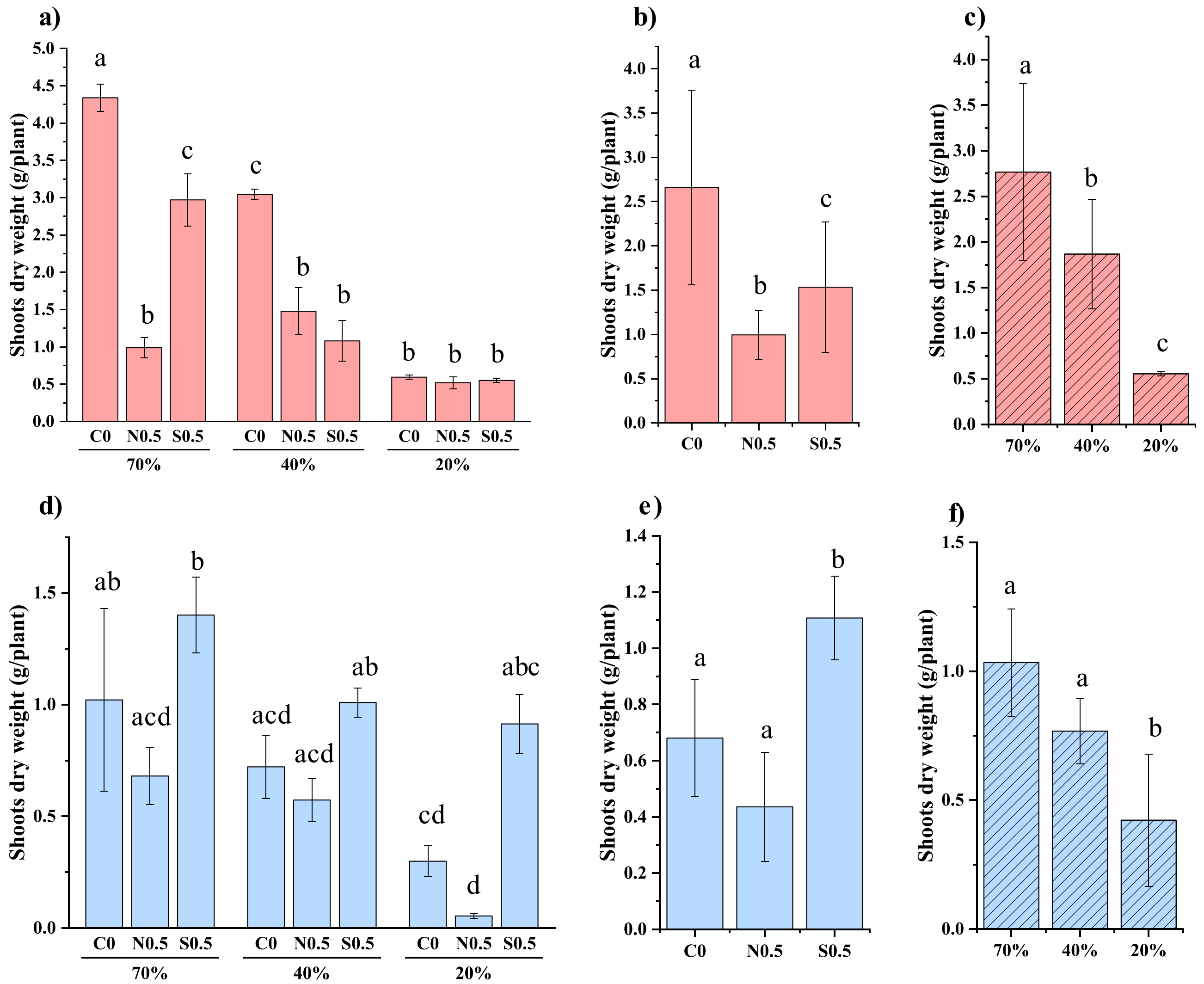

Figure 5. SDW of spinach grown in two soil types. For the CL soil (in red), (a) SDW under different levels of SM and treatments, (b) SDW in treatments irrespective of SM, and (c) SDW in SM irrespective of treatment. For the SD soil (in blue), (d) SDW at different levels of SM and treatments, (e) SDW in treatments irrespective of SM, and (f) SDW in SM irrespective of treatment. Values are reported as mean ± standard deviation ($n = 5$). For each plot, means followed by the same letter were not significantly different at the $p \leq 0.05$ level (Tukey's HSD). (a) and (d) highlight the difference at the two-way interaction (treatment × soil moisture). See Table 2 for details of ANOVA results. Here, treatments are referred to as control (C0), nanocellulose at 0.5 wt % (N0.5), and commercial superabsorbent at 0.5 wt % (S0.5).

and surface area of nanocellulose SAPs[32] very likely accelerated the biodegradation process by easing the microbial access to the carbon source. This effect released all the water taken up by the superabsorbent to the soil, inducing a higher waterlogging effect and causing a breakdown in the soil structure which remained throughout the whole study. Supporting previous studies, our findings suggest that the optimal concentration of SAPs varies depending on soil texture, dispersibility, and superabsorbent characteristics. SAPs should only be employed in areas with severe water stress.[13,46]

On the other hand, for SD soils, the SDW significantly increased with the application of a commercial SAP. This is observed at all levels of SM which confirms the suitability of the nanocellulose SAP as a water retention agent promoting plant growth under water stress. The SDW in soil amended with the nanocellulose SAP was slightly lower than that of the control at all levels of SM. Though this decrease was not low enough to represent a significant difference, the reduction in plant growth can be associated with a nitrogen deficiency caused by the immobilization of nitrogen.[49] This occurs when large amounts of labile carbon (C) are added to soils, thereby increasing the activity of the soil microbial community. As a result, soil microbes use the available nitrogen in the soil for microbial growth and activity, limiting the nitrogen available for plant growth. The immobilization of nitrogen is further accentuated when lignocellulosic sources of highly labile carbon content, such as pine bark or sawdust, are added to soils.[49,50] The significant increase in soil respiration rates with application of nanocellulose SAPs indicates an increase in the size of the microbial community which likely led to nitrogen drawdown. Immobilization of nitrogen appears in treatments with carbon/ nitrogen (C:N) ratios higher than 25:1.[51,52] For SD soil, the C/N molar ratio increased from 24 to 28 upon the addition of nanocellulose SAP; this represents a high C/N ratio at which immobilization of nitrogen occurs[51,52] (Table 3).

Soil respiration rates are also related to the superabsorbent degradation in soil, where nanocellulose SAP degradation had a half-life of 5 and 7 days for the CL and SD soils, respectively. The greater decomposition in the CL soils is due to their

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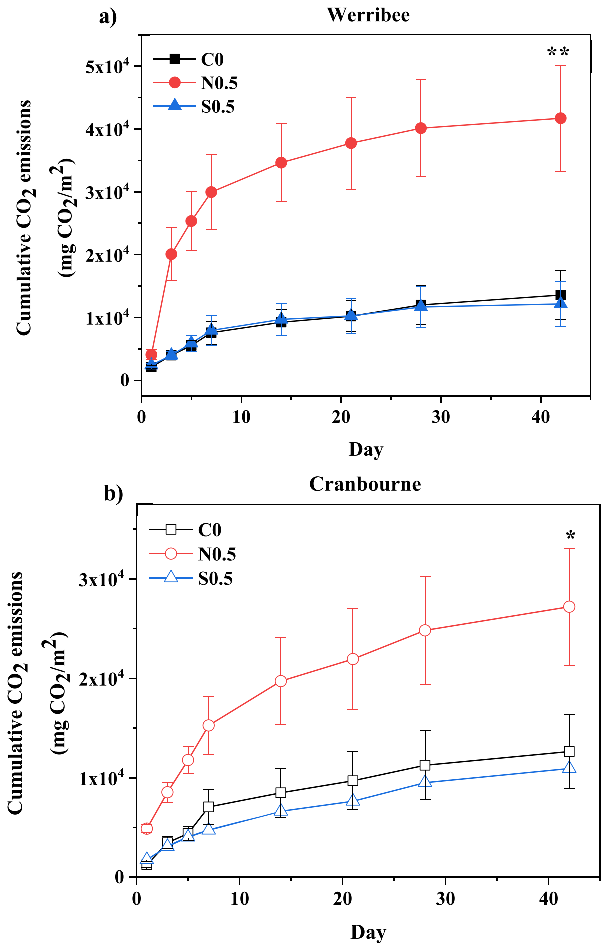

Figure 6. Cumulative CO_2 emissions of different soil treatments for: (a) CL soil and (b) SD soil. Results are reported as mean ± standard deviation ($n = 5$). The asterisks above each data point show the level of significant difference compared to the control, C0. Here, * is $p \leq 0.01$, and ** shows $p \leq 0.001$.

Table 3. Total Carbon (%), Nitrogen (%), and C/N Molar Ratio of Soil Treatments Measured by Elemental Analysis[a]

treatment	total carbon (%)	total nitrogen (%)	C/N molar ratio
CL soil			
C0	1.3	0.13	12
N0.5	1.5	0.13	14
SD soil			
C0	1.2	0.056	24
N0.5	1.4	0.056	28

[a]Here, treatments are referred to as control (C0) and nanocellulose at 0.5 wt % (N0.5).

higher amount of organic matter, able to support a larger microbial community with a greater ability to decompose nanocellulose than in SD soil.[53] This rate of decomposition supports previous studies reporting the degradation of cellulose to mostly occur within 16 to 30 days.[54,55] The rate of cellulose breakdown varies between soils and environmental conditions (moisture and temperature),[56] as observed in this study.

■ CONCLUSIONS

This study evaluated the effect of nanocellulose SAPs on the growth of spinach. Two soils, one a CL and the other a SD soil (SD), were amended with the nanocellulose SAP, and spinach plants were grown at different levels of SM to simulate water-stress conditions. Soil water retention, porosity, WP, and plant biomass were measured, and the results were compared with those of soils treated with a commercial anionic polyacrylamide-based SAP. The biodegradation rate of the nanocellulose superabsorbent was determined and related to the soil microbial activity.

For both soils, porosity increased with the application of a superabsorbent, independent of the type of SAP. This effect was more accentuated in CL soils. Soil water retention significantly increased with the application of the commercial superabsorbent, being approximately 170 and 220% times more than the control for the CL and the SD soil, respectively.

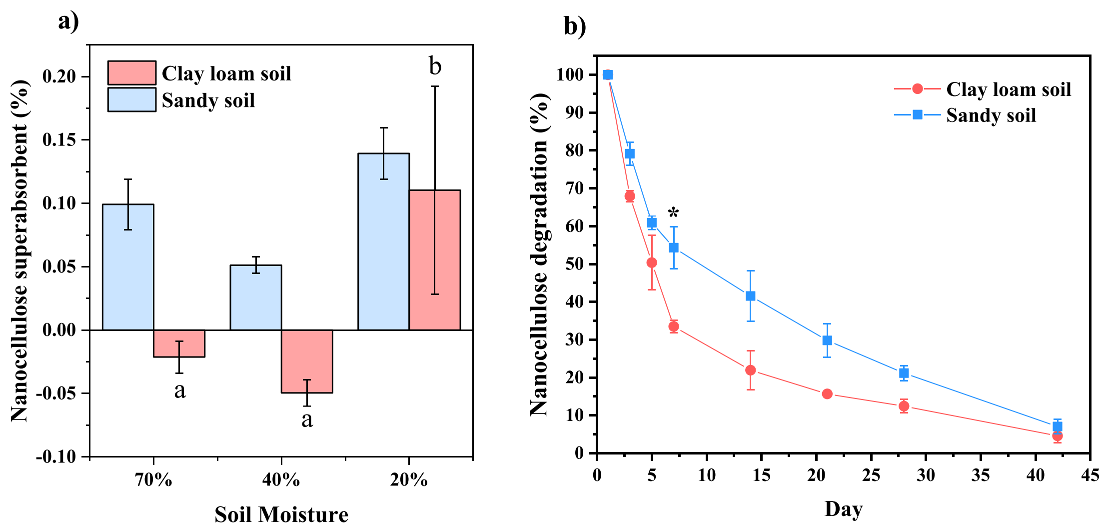

Figure 7. (a) Effect of the soil type on the biodegradation of the nanocellulose SAP over time. (b) Amount of nanocellulose superabsorbent remaining at the end of the plant experiment for both soils. Results are reported as mean ± standard deviation ($n = 5$). Bars followed by the same letter were not significantly different at the $p \leq 0.05$ level (Tukey's HSD). The asterisk above day 7 in (b) shows the level of significant difference at the $p \leq 0.05$ level.

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For both types of superabsorbents, the increase in water retention was lower in the CL soil. This is attributed to the effective CEC which is about eight times higher in the CL soil.

The effect of superabsorbent application on WP and plant biomass varied depending on the soil type. For the SD soil, WP and plant biomass were the highest in treatments with the commercial superabsorbent, independent of SM. For the CL soil, WP and plant biomass were significantly lower in treatments with superabsorbent compared to the control. This effect was magnified in treatments with a nanocellulose superabsorbent and is likely attributed to waterlogging and the fast biodegradation of this SAP, where about 50% of the initial mass remained after 5 days of exposure in this soil. Immobilization of nitrogen by the increasing microbial activity may have also contributed. This is supported by the significant increase in soil respiration rates when the nanocellulose SAP is used in both types of soil. This study identifies the commercial SAP to have a superior performance compared to the nanocellulose superabsorbent. There is a need to overcome the waterlogging stress induced by SAP application. This can be achieved by adjusting the application rate of superabsorbents depending on the type of soil. In addition, modification of the nanocellulose SAP physico-chemical structure is necessary to inhibit its biodegradation and fully increase its benefits for agricultural use.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.1c00178.

Characteristics of the soils selected for the experiments (PDF)

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Notes

The authors declare no competing financial interest.

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