

Residual effect of bentonite-humic acid amendment on soil health and crop performance 4-5  
years after initial application in a dryland ecosystem

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**Abstract:** Degraded soils causing from natural and human affects are universal in arid and semi-arid regions all over the world. Bentonite and humic acid (BHA) are increasingly being tested to remediate these degraded lands with potential benefits on crop production and soil health. The objective of this paper was to determine the residual effects four to five years after a one-time BHA application at six rates on (i) dynamic changes in soil properties, and (ii) oat crop productivity parameters, in a dryland farming ecosystem. With increasing rates of one-time BHA application, soil profile water storage displayed a piecewise linear increase plus plateau, whereas soil electrical conductivity, pH and bulk density were all reduced significantly ( $P < 0.05$ ) in the 0-20 cm and 20-60 cm layers. The improved soil environments gave rise to an increased activity of soil enzymes urease, invertase and catalase that respectively reached the peak values of 97%, 37% and 32% at the rates of 21 to 24 Mg BHA ha<sup>-1</sup>. These conversely boosted soil nutrient turnover, leading to a 40% higher soil available P. Compared with the control treatment, application of BHA at the estimated optimum rate (roughly 24 Mg ha<sup>-1</sup>) increased grain yield by 20%, protein yield by 62%, water use efficiency by 41%, and partial factor productivity of N by 20%. Results of this study showed for the first time that a one-time BHA application would be a new and effective strategy to combat land degradation, drought, and promote a sustainable soil micro-ecological environment in dryland agroecosystem under a varying climate scenario.

**Keywords:** Sustainable dryland farming; clay soil amendment; soil water use; organic matter; enzyme activity; nutrient turnover

## 1. Introduction

Globally, soil degradation resulting from changing climate scenarios and poor farming practices affects more than 30% of earth's land surface [1], reducing food production security and increasing irrigation demand in dryland farming regions [2]. Various soil amendments have been evaluated to alleviate degraded soil in dryland regions. The performance of soil amendment applications such as clay and biochar varies among land uses, soil types and ecosystems of the world. Bentonite, an aluminum phyllosilicate clay consisting predominantly of montmorillonite, can be used as a natural and non-toxic water absorbing soil amendment [3]. Bentonite has been widely used as a lubricant in drilling mud and boreholes, to stabilize soil structure in construction sites [4], to improve soil rheological or sealing properties in geo-environmental applications, and for heavy metal absorption and fixation in waste water treatment [5]. Some studies on the use of bentonite as an agricultural soil amendment demonstrated a significant improvement in soil water storage [3] and reduction in soil bulk density [6] in the 0-60 cm soil profile in fragile semi-arid agricultural areas.

Humic acid (HA) is a chemical constituent of soil organic matter [7]. Previous pot studies found that HA application with irrigation water had a positive effect on oat (*Avena nuda* L.), winter rapeseed (*Brassica napus* L.) and leek (*Allium ampeloprasum* L.) root and shoot growth [8-10]. Application of a combination of bentonite with humic acid (BHA) has been evaluated as a new water saving and ecological restoration strategy on soils with low clay and organic matter in the arid and semi-arid region of northern China [6].

The valuable role of BHA application on soil quality may comprise the mitigation of soil

compaction, enhancement of water retention, and their combined impact on soil microorganisms [11]. BHA has the potential to be used as an adaptive strategy to improve soil microbiological properties in both the top and deeper layers [12]. Some studies have investigated the responses of soil microbiological properties to changes of soil physicochemical properties in the surface soil layers, with the application of bentonite amendment [13, 14]. Currently, climate change is leading to an increase in the extent of temperate drylands, intensifying drought events and reducing plant and microorganism available water in deeper (>20 cm) soils during the crop growing season [15]. However, there is still a lack of information on the amendment effect on soil enzyme activities and consequently nutrient availability in the soil profile. Soil enzyme activity has been quoted as a viable bio-indicator of soil microbiological activity because of its greatly sensitive response to spatial variations of soil health [16]. Soil enzymes (catalase, invertase and urease) play a vital role in soil ecosystems and contribute to global soil organic matter turnover and nutrient cycling throughout the soil profile [11, 17]. Hence, data on enzyme activity as soil microbiological index treated with BHA or other similar materials might supply a beneficial insight into the contribution of the natural water-absorbing amendments to soil quality enhancement.

Changes of soil physicochemical characteristics for instance soil pH and nutrient status following amendment treatments of biochar and manure were not always discovered [16, 18]. Effectiveness of BHA on enhancing soil quality, however, depends on a number of factors including soil texture, application rate and accompanying soil and crop management [19]. It has been suggested that excessive tillage and repeated irrigations may degrade the

effectiveness of bentonite [3, 6, 20]. On the other hand, bentonite and HA are extremely resistive to microbial degradation, which implies that annual reapplication of BHA might not be essential, although the effectiveness of BHA may diminish over time. Residual effects of bentonite application on soil water-holding capacity and water status have been showed in a few field studies [3, 6]. Rollins and Dylla estimated that the effectiveness of a single bentonite application declined at a rate of about 14-20% per year, but this varied with frequency of damage by wind erosion and wetting and drying cycles [20]. In contrast, the long-term impacts of manure and biochar, both deeply studied soil amendments, are uncertain to be related to the short-term effects on soil physicochemical and microbiological performance [16]. The difference is a result of the aging processes and the development of unbalanced conditions in chemical exchange and biological activity in the soil amendment systems [21]. However, the information is still scarce on residual effect over time of a one-time BHA application.

Clay amendment induced soil quality improvements have been shown to promote the establishment of agricultural field crops such as maize (*Zea mays* L.), millet (*Panicum miliaceum* L.) and squash (*Cucurbita pepo*) [3, 6, 22]. However, the impact of clay application on soil nutrient cycling and enzyme activity has not been fully tested in soil-crop agroecosystems.

Oat (*Avena nuda* L.) is gradually being exploited for human consumption as a valuable health-food worldwide [23-25]. It has generally been regarded a low-input cereal crop and has been conventionally grown on farms with low soil fertility as well as moderate or low management [24]. Meanwhile, oat has an acute sense of water stress that occurs with

different and unpredictable frequencies and length across growing season [26, 27]. Climbing water constraints in deep soils with the rise of drought are severely curtailing productivity of oat and other grain cereal crops [28]. One potential method of managing the risk of oat crop losses in dry seasons, and optimizing the benefits of favorable seasons is the application of BHA [6] that both conserves water and releases nutrients into their available forms [9].

We assumed that the effects of a single low-rate BHA application on crop water use efficiency and soil water storage might be ephemeral because of the breakdown of HA and the loss of bentonite through wind erosion and possible dilution by tillage or transport to deeper depths by water percolation through the soil profile during heavy storms. In contrast, a higher rate of BHA application may lead to a longer-term improvement of soil enzyme activity, water storage, and nutrient cycling throughout the soil profile, and therefore, result in a positive effect on crop productivity even at 4-5 years after application. To examine this hypothesis, a 5-year field experiment with oat crop was conducted in a typical dryland farming area to compare one-time application of BHA at six rates.

The specific aim of this research was to assess the effectiveness of a single BHA application at different rates for improving (i) soil health indicators (soil water storage, nutrient availability, and enzyme activities), (ii) oat crop performance parameters (grain yield, protein, partial factor productivity of nitrogen and water use efficiency) at 4 and 5 years after the initial application, and (iii) determine the optimum rate of one-time BHA application all in a dryland farming ecosystem.

## **2. Materials and methods**

### *2.1. Study site*

The experiment was conducted from 2011 to 2015 at the Yijianfang village of Qingshuihe County Research Centre, Hohhot, Inner Mongolia, in northcentral China (39.5 °N, 111.7 °E, ca. 1374 m above sea level). The experimental site has a sandy loam texture. The average annual rainfall is 365 mm. Further detail on the soil properties and climate can be found in O'Connor, Hoang (29).

The region is part of the Loess Plateau along the Great Wall in north central China where main broad group of soil formed is the Huangmian soil (Calcic Cambisols in the FAO soil classification system) ranging in depth from 30 to 80 m. Zhu, Li (30) provided a detailed description of the soils of the Loess Plateau in China.

## *2.2. Characteristics of BHA soil amendment*

The commercial BHA amendment was a mixture of bentonite, humic acid (HA), Na<sub>2</sub>CO<sub>3</sub> and cellulose with a ratio of 91:6:2:1 as described in Zhou, Monreal (6). Briefly, bentonite was mined from a local source by Tirock Co., Ltd., Naiman County, Tongliao, Inner Mongolia of China. It contained (wt/wt): 73.2% SiO<sub>2</sub>, 11.4% Al<sub>2</sub>O<sub>3</sub>, 2.67% CaO, 2.58% K<sub>2</sub>O, 1.05% MgO, 0.31% Na<sub>2</sub>O, and 0.29% Fe<sub>2</sub>O<sub>3</sub>. Chemically, the free HA fraction occupies 38.3% (wt/wt) of the total HA content. The BHA mixture is marketed to local farmers as a soil amendment for coarse-textured sandy soils.

## *2.3. Experimental set-up and field management*

The experiment consisted of six treatments and carried out as a randomized complete block (RCB) design with three replications under the condition of no irrigation, depending upon natural rainfall. The treatments were (1) control or check with no applied BHA, and application of BHA at rates of (2) 6 Mg ha<sup>-1</sup>, (3) 12 Mg ha<sup>-1</sup>, (4) 18 Mg ha<sup>-1</sup>, (5) 24 Mg ha<sup>-1</sup>

and (6) 30 Mg ha<sup>-1</sup>. The BHA was broadcast uniformly on the soil surface and incorporated into the 0-15 cm soil profile by rotary cultivator, before planting on May 2011. All plots, with or without BHA application, received the same management practice of annual spring cultivation at about 15 cm depth with a rotary cultivator. Each plot had an area of 6 m×5 m.

Oat seeds were sown at the depth of approximately 4 cm in late May and harvested in mid-September in each year (2011-2015). Each plot consisted of 20 rows of oat crop with row spacing 25 cm, and sowing density of 375 plants m<sup>-2</sup>. In each year, fertilizer N as urea at 34.5 kg N ha<sup>-1</sup> and P as diammonium phosphate (containing 27 kg N ha<sup>-1</sup> and 69 kg P ha<sup>-1</sup>) were broadcast and incorporated in all plots prior to seeding. Additional urea (69 kg N ha<sup>-1</sup>) was manually applied at GS 31.

The date of the key phenological period when 50% of the oat crop reached a specific stage was recorded per plot

The dates of key phenological stages were recorded per plot when 50% of the oat crop achieved the certain stage, according to Zadoks' scale. These contained seedling (GS 12), jointing (GS 31), heading (GS 55), grain filling (GS 75) and maturity (GS 92) [29]. The experiment followed local management recommendations for weed control as well as other agronomical practices.

#### *2.4. Sampling and measurements*

We sampled soils five times during the growing season (GS 12, GS 31, GS 55, GS 75 and GS 92) in 2014 and 2015. Samples collected three replicates for each treatment that selected midway between two rows within each plot. After removing any surface visible debris, a 5 cm -diameter handheld auger was used to collect soil samples at depths of 0-10, 10-20, 20-40,

40-60, 60-80 and 80-100 cm. The cores from each plot were combined to obtain one composite sample for each layer (i.e. 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm). Each soil sample was thoroughly mixed, homogenized, and then divided into two parts. One part was air-dried, sifted through a sieve (2-mm) and stored for farther tests of soil electrical conductivity, pH, alkaline nitrogen (AN), phosphorus (AP), and potassium (AK), soil organic matter. The second part was instantly stored in a cooler (4 °C), and assessed for the activities of catalase, invertase and urease within two weeks after soil sample collection.

#### *2.4.1. Determination of soil physicochemical properties*

Soil bulk density (SBD,  $\text{g cm}^{-3}$ ) was measured using a 10 cm diameter and 5 cm high cutting ring. Soil electrical conductivity (SEC,  $\text{mS m}^{-1}$ ) and pH were determined in suspensions of distilled water and soil (5:1), respectively using hand-held conductivity and pH meters. Soil water content (SWC, %) was determined by oven-drying (105 °C) samples until constant weight. Soil water storage (SWS, mm) at each layer was evaluated according to Eq. (1):

$$SWS(mm) = SWC(\%)/100 \times \rho_b(g\text{ cm}^{-3})/\rho_w(g\text{ cm}^{-3}) \times d(mm) \quad (1)$$

where SWC is soil water content,  $\rho_b$  is soil bulk density,  $\rho_w$  is water density and  $d$  is soil depth.

Soil available phosphorus (AP), alkaline nitrogen (AN) and available potassium (AK) were quantified by  $\text{NaHCO}_3$ -Molybdenum antimony colorimetric, NaOH alkali diffusion, as well as  $\text{NH}_4\text{OAc}$ -flame photometry methods, respectively; Soil organic matter (SOM) was measured by oxidation with the  $\text{K}_2\text{Cr}_2\text{O}_7$  and exothermic heating method [31].

#### *2.4.2. Soil enzyme activities*

Soil urease activity was determined by measuring the reduction of urea following incubation [32]; the urease activity was calculated and expressed as  $\mu\text{g NH}_3\text{-N g}^{-1}\text{ soil (24 h)}^{-1}$  [33].

Soil invertase activity was assayed according to Guan, Zhang (32), by monitoring consumption the of sucrose following incubation; invertase was expressed as  $\text{mg glucose g}^{-1}\text{ soil (24 h)}^{-1}$  [34].

Soil catalase activity was determined according to Guan, Zhang (32), by measuring the reduction of  $\text{H}_2\text{O}_2$  for soil suspended in a reaction solution; the catalase was expressed as  $0.1\text{ mol L}^{-1}\text{ KMnO}_4\text{ ml g}^{-1}\text{ soil (30 min)}^{-1}$  [35].

#### *2.4.3. Plant productivity and grain protein*

To measure plant aboveground biomass yield (BY) and grain yield (GY) the two  $1.0\text{ m}^2$  areas of each plot was harvested in 2014 and 2015 after the oat reached physiological maturity. Grain samples of each plot (5 g) were pulverized to pass through a 1-mm mesh screen. After being dried at  $75\text{ }^\circ\text{C}$  until constant mass, a subsample of around 1 g was counted and digested for the assessment of total N content, using the Kjeldahl digestion as well as analytical method. Crude protein (GP) was determined by multiplying the grain N concentration by 6.25 [36]. Total grain protein yield (TGP) was estimated as the product of GY and GP concentration. Total growing season evapotranspiration (ET, mm) was calculated according to Wang, Wei (37). Water use efficiency (WUE,  $\text{kg ha}^{-1}\text{ mm}^{-1}$ ) was calculated from GY and ET [37]. Partial factor productivity of nitrogen (PFPN) was calculated by dividing the GY by applied N fertilizer [38].

#### *2.5. Data analysis*

One-, two- or three-way analyses of variance (ANOVA) were performed, respectively for one-time point variables (e.g. yield, protein), multi-time point variables (soil nutrient dynamics), and variables involving soil depth and growing season (e.g. enzyme activity), with the MIXED procedure of SAS, using the Type III and REML estimation methods (SAS Institute Inc., Cary, NC, USA). Rate of one-time application of BHA in 2011 was considered as a continuous variable for analyzing trends, and the measured dependent response variables were fitted to piecewise linear plus plateau models using the NLIN procedure in SAS. The BHA rate at which a plateau was reached was considered the optimal rate for the measured dependent response variable; additional BHA would not increase the response variable. Redundancy analysis (RDA) methods based on the ggvegan package of the R software (R Core Development Team, R Foundation for Statistical Computing, Vienna, Austria) were performed to elucidate the relationships among soil parameters and plant characteristics.

### **3. Results**

The ANOVA showed that BHA treatment, soil depth, growth stage, year and their interactions had a highly significant effect ( $P < 0.01$ ) on all of the soil parameters measured at each depth and growth stage (SWS and three enzyme activities), soil parameters measured once a year (at GS 92) at different depths (SEC, pH, SBD AN, AP, AK and SOM), and crop parameters measured once per year (GY, TGP, ET, WUE, PFPN and BY).

The relationships between all of the measured parameters and BHA treatment rate displayed a linear or piecewise linear plus plateau nature. The values reported in the results section are the parameters of the linear or piecewise linear models of the respective dependent variable against level of BHA treatment rate. The slopes of the linear sections have

units of change in the dependent variable per Mg BHA ha<sup>-1</sup>; the units of the plateaus were the dependent variable at the plateau level of BHA. The term plateau is used to describe the region of no further change in the dependent variable with further increase in BHA rate; depending on the dependent variable, plateaus occurred following an increase or decrease of the dependent variable.

### 3.1. Soil physicochemical properties

The SWS displayed a significant piecewise linear (slope plus plateau) shape against BHA application rates (**Fig. 1A**), with the slopes varied from 0.05 to 0.27 mm / (Mg BHA ha<sup>-1</sup>) in 2014 and from 0.03 to 0.19 mm / (Mg BHA ha<sup>-1</sup>) in 2015 (**Table 1**). The response curves did not always show plateaus, but when there was a plateau, it often started at the BHA application rate of about 24 Mg ha<sup>-1</sup> (range of 22 to 29 Mg BHA ha<sup>-1</sup>). On average, SWS increased by 12 to 29 % over the no BHA control treatment.

At the 0-60 cm soil layer, SEC, pH and SBD displayed a decreasing trend with increasing BHA application rates in both 2014 and 2015, although the decline rates varied among soil depths and between the two years (**Fig. 2A-C**). The most effective response often occurred at the estimated application rate of 21 Mg ha<sup>-1</sup> (ranging from 7 to 30 Mg BHA ha<sup>-1</sup>). At this rate, the corresponding reduction was up to 34 % for SEC, 5% for pH and 12% for SBD, compared with the no BHA treatment.

### 3.2. Enzyme activity

The enzyme activities generally tracked SWS in both growing seasons (**Table 1** and **Fig. 1 B-D**). Specifically, with each Mg BHA ha<sup>-1</sup> of application, the improved activities ranged from 5 to 30 µg NH<sub>3</sub>-N g<sup>-1</sup> soil (24 h)<sup>-1</sup> for urease, from 0.1 to 0.5 mg glucose g<sup>-1</sup> soil (24 h)<sup>-1</sup>

for invertase, and from 0.05 to 0.19 mol L<sup>-1</sup> KMnO<sub>4</sub> ml g<sup>-1</sup> soil (30 min)<sup>-1</sup> for catalase, respectively (**Table 1**).

Plateaus in enzyme activities were reached more often than in SWS (**Table 1** and **Fig. 1**). In all cases, the highest enzyme activity occurred at approximately 24 Mg BHA ha<sup>-1</sup> (ranging from 16 to 28 Mg BHA ha<sup>-1</sup>). At this rate, the highest corresponding increase compared to the control was up to 97% for urease, 39% for invertase and 32% catalase in the wet year (2014).

Overall, BHA application showed a large effect on most of the enzyme activity parameters, particularly in the 20-40 cm soil layer, during the mid- to late-growing season. It corresponded to the rapid crop development stages in both 2014 and 2015.

### 3.3. Soil available nutrient

In both 2014 and 2015, soil nutrient concentrations and SOM generally tracked SWS (**Fig. 3A-D**), with increment rates varied from 0.4 to 0.9 mg kg<sup>-1</sup> for AN, 0.1 mg kg<sup>-1</sup> for AP, 0.4-1.8 mg kg<sup>-1</sup> for AK, and 0.03-0.14 g kg<sup>-1</sup> for SOM, with each Mg BHA ha<sup>-1</sup> of application. Plateaus were not reached for AN, and only sometimes for AP, AK and SOM. When there was a plateau, it often occurred at the rate of about 20 Mg ha<sup>-1</sup> (range 5 to 30 Mg BHA ha<sup>-1</sup>). At this rate, the corresponding increases were up to 44 % for AP, 17% for AK and 45% for SOM, compared with the control.

### 3.4. Yield and grain protein

Over the two growing seasons, GY, TGP and BY (**Fig. 4A, B and F**) were all influenced by BHA treatments. There was a general linear increasing trend, with the slopes of 22 kg GY ha<sup>-1</sup> and 8 kg TGP ha<sup>-1</sup> in both 2014 and 2015, while the slope of BY was 54 kg ha<sup>-1</sup> in 2014 and 48 kg ha<sup>-1</sup> in 2015. The estimated highest GY, TGP and BY were reached, respectively at

22, 24 and 24 Mg BHA ha<sup>-1</sup> in 2014 (a wet year), and at 25, 26 and 27 BHA Mg ha<sup>-1</sup> in 2015 (a dry year). At this rate of BHA application, GY, TGP and BY were respectively 17, 53 and 15% in 2014, and 20, 62 and 15% in 2015, greater than the control.

### 3.5. *Water and nitrogen use*

With increasing the rate of BHA application, WUE was increased linearly with the slope of 0.2 kg yield ha<sup>-1</sup> mm<sup>-1</sup> in both 2014 and 2015 (**Fig. 4C-E**). PFPN displayed the same response trend as the WUE, While ET showed the opposite trend, with the corresponding reduction of ET at 3.1 mm in 2014 significantly greater than the 1.4 mm in 2015 (**Fig. 4C**). At the estimated shoulder application rate of 16 and 19 Mg BHA ha<sup>-1</sup>, the WUE was enhanced by 41% and ET was reduced by 17% in the wet year, compared to the control (**Fig. 4C and D**). The variation of PFPN (**Fig. 4E**) was consistent with GY.

### 3.6. *Relationships between crop performance parameters, soil physicochemical properties and enzyme activity*

There were highly significant ( $P < 0.01$ ) correlations among the crop performance parameters (GY, TGP, BY) and soil health parameters (SWS, urease, invertase, catalase, nutrients, SOM). Soil chemical and physical parameters SEC, pH, and SBD also displayed highly significant correlations with each other, and correlated with other soil health parameters, soil water storage, available nutrients and enzyme activity, and with crop performance parameters. TGP had the closest correlation with the activity of soil catalase in 2014, and with AN and AP in 2015. BY and GY were more closely correlated with urease, AN and AP in both 2014 and 2015 (**Fig. 5**). Bi-plots from the redundancy analysis (RDA) showed a consistent trend where increasing rates of the BHA application increased the

magnitude of RDA1 (**Fig. 5**), demonstrating that application of BHA created a healthier soil environment and improved crop performance.

## 4. Discussion

### 4.1. *Impact of BHA on soil health parameters*

Increase in SWS was deemed the most important effect of the BHA application, and it influenced other soil and crop response parameters. In this study, we demonstrated a significant effect of BHA at the 4-5 years after its initial application, and such effect was greater in 2014 (a wet year) than in 2015 (a dry year) (**Table 1** and **Fig. 1**). There was also a significant improvement on SWS in the 0-20 cm soil layer and at the deeper depths (20-60 cm) and corresponding reduction of water loss out of the rooting zone to the ground water (**Table 1**). The results of this study indicate that there was likely a significant amount of BHA remaining in the soil. The greater improvement in SWS of the deep soil layer, notably in the wetter year (2014) (**Table 1**), was mainly attributed to the sufficient rainfall during the growing season to supply the continuous water uptake by the crop with the excess percolating through the rooting zone to deeper depths. To a lesser extent, the increased SWS may have reflected the corresponding retention of soil water from previous years (e.g., annual rainfall was 549.6 mm in 2013 and 456.2 mm in 2014). Some earlier studies on the long lasting impact of BHA (“tiny reservoir”) application on sandy soil water storage [6, 39] support our conjecture. At the high rates of application, BHA in the soil could absorb a large amount of water, and suppress surface ineffective evaporation [40], resulting in reduced evapotranspiration (**Fig. 4C**). With the infrequent and minor magnitude rain events at the test site, loss of the BHA by erosion, or leaching would be diminished and more BHA would

remain intact particularly deeper in the soil profile. This would foster greater and longer soil water retention in our semiarid region than in other sites where greater rainfall or different soil textures and pedogenesis have diminished the effectiveness of the applied BHA in a few years after application [6].

Improvements in other soil parameters including the reduced SEC, pH and SBD and increased SOM could all be attributed to improved overall soil health [15, 41, 42]. Collectively, the increased SOM and improved soil physicochemical properties could provide a healthy and suitable living condition for soil microorganisms [11, 43]. Improvement in soil microorganisms likely contributed to more timely and efficient nutrient turnover and cycling, leading to higher plant available N, P and K concentrations [44-46]. The lower SBD in both the layer of incorporation (0-20 cm) and adjacent deeper layers (20-60 cm) (**Fig. 2D**) was likely associated with the migration of BHA to subsurface soil layers, which may have helped the extension of oat root exudates and root mycorrhizae deeper in the soil profile, thereby increasing the binding of soil particles [47]. This indicates the importance of physicochemical adjustment for soil micro-ecological environment improvement in the dryland agroecosystem.

#### *4.2. Plant response to BHA*

In this field study, we demonstrated a clear link between BHA induced improvements in SWS and other soil health indicators (enzyme activity and available soil nutrients) and grain yield and grain protein (**Fig. 5**). The improved crop response to BHA in both wet and dry years was likely both a direct effect of the improved plant-water status and nutrient uptake due to the increased transpiration [48], and an indirect effect through an improvement in soil

health parameters such as increased microbial activity that led to increased plant available nutrients [11]. Increasing BHA application rates resulted in higher yields (**Fig. 4**). This in turn, led to the improved both water use efficiency (WUE) and partial factor productivity of nitrogen (PFPN) in both wet and dry years (**Fig. 4D and E**).

#### *4.3. Optimization of the BHA application rate*

In this study, some of the dependent variables exhibited a linear response to the rate of BHA application, with a plateau, while others exhibited only a linear response. The absence of a plateau indicated that the maximum response had not yet been achieved with the maximum BHA rate of 30 Mg ha<sup>-1</sup> used in our experiment. However, it has been noted that too much BHA would likely be detrimental as demonstrated in an earlier study [49], where failure of plant growth was found with large amounts of bentonite application in mining areas. In this study, 24 Mg BHA ha<sup>-1</sup> was identified to be the lower end of the optimum range while the maximum rate of 30 Mg BHA ha<sup>-1</sup> appeared not to reach the upper limit of the optimum range for some of the soil and plant growth parameters. Ideally, BHA rates in an experiment would be distributed equally on either side of the optimum. The failure of identifying the plateaus of soil health indicators in the dry year (2015) appeared to indicate that higher rates of BHA may be required, or it may simply reflect the reduced effectiveness of BHA due to the limited water under the adverse conditions. Therefore, future experiments including higher rates of BHA may help to more clearly identify the optimum application rate.

#### *4.4. Longevity of BHA*

Bentonite is a stable mineral which could be lost by erosion or downward migration through the soil profile while the HA is organic and could be decomposed. Rollins and Dylla

estimated that the effectiveness of bentonite for sealing dams declines at a rate of  $\sim 20\%$  year<sup>-1</sup> due to the loss by wind and water erosion, which would indicate no residual effect after five years [20]. Although the rate of degradation/disappearance of BHA after its application was not measured in this study, we demonstrated a substantial residual effect at 4-5 years after the BHA application. This reflected that our test condition (incorporation in the soils) was suitable for the semiarid region and is in contrast to the estimated rate of BHA broken down or lost under the wet conditions by Rollins and Dylla [20]. If the rate of loss of effectiveness was known, then appropriate recommendations could be made for farmers to reapply a portion of the BHA after several years to ensure its continued function in improving soil and agroecosystem services.

The rate at which the effectiveness of BHA declines is a function of the site's climate, soil characteristics and ecosystem, and deserves further study. Other soil amendments, e.g. biochar and manure, can be used for similar purposes in improving crop productivity but the availability of biochar is limited in our study area. Li, Xiong (50) suggested an optimum biochar application rate of around 30 Mg ha<sup>-1</sup> (roughly 4761 USD ha<sup>-1</sup>), which is about 15 times the cost of a single 30 Mg ha<sup>-1</sup> (317 USD ha<sup>-1</sup>) application of BHA. On the other hand, the component of biochar is stable and may only require a single application.

## 5. Conclusions

For the first time, we demonstrated that one-time BHA application improved and sustained better agricultural ecosystem balance and crop productivity. This was achieved through effective soil hydrologic regulation, soil enzyme activity and nutrient turnover in topsoil and subsoils, thereby leading to the efficient use of soil water and nutrient by the oat crop, and the

corresponding increases in grain protein, grain yield, water use efficiency, and partial factor productivity of nitrogen. The improvements measured four to five years after the initial application were linear with the BHA application rates, and many, but not all, response parameters reached a plateau or saturation point at approximately 24 Mg ha<sup>-1</sup>. Our study implies that one-time BHA application may serve as a new sustainable strategy to improve soil micro-ecological environment and crop productivity and to cope with global climate change in arid and semi-arid regions. However, it should be pointed out that a successful strategy for drought abatement in one crop-, soil- and site-specific agroecosystem may have little effect in other soils and ecosystems; this aspect needs further study. Further research at higher application rates is also required to better define the optimum application rate, to measure the degradation and long-term stability of the BHA amendment, and to determine the economics under variable weather conditions.

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**Table 1**

The parameters of the 2014 and 2015 linear or piecewise linear models of soil water storage (SWS), urease, invertase and catalase activity in different depths plotted against one-time bentonite-humic acid (BHA) application rate.

		Intercept		Slope*		Plateau initiation	
		2014	2015	2014	2015	2014	2015
SWS	0-10	10.2	6.8	0.05	0.03	24	-
	10-20	9.5	8.6	0.10	0.07	24	29
	20-40	21.3	16.7	0.27	0.19	22	23
	40-60	18.7	15.5	0.18	0.09	-	-
Urease	0-10	0.83	0.98	0.013	0.020	24	20
	10-20	0.67	0.72	0.017	0.030	24	16
	20-40	0.39	0.33	0.016	0.008	24	-
	40-60	0.35	0.28	0.011	0.005	24	-
Invertase	0-10	24.9	21.0	0.33	0.33	-	-
	10-20	17.9	15.9	0.18	0.50	-	-
	20-40	7.5	5.5	0.12	0.19	24	-
	40-60	6.5	5.3	0.07	0.12	-	-
catalase	0-10	15.7	13.9	0.08	0.11	24	-
	10-20	14.5	12.8	0.11	0.12	-	-
	20-40	12.5	10.7	0.12	0.12	23	24
	40-60	12.7	10.7	0.13	0.10	-	-

\* Slopes of the regression lines were highly significant ( $P < 0.01$ ) for all parameters. Units of intercept are the units of the corresponding dependent variable; units of slope are quotient of dependent variable and rate of BHA; units of intersection are  $\text{Mg BHA ha}^{-1}$ . Plateau initiation for models that did not converge to piece-wise linear are indicated by “-”.

## Figure legends

**Fig. 1** Means (of all growth stages) of A) soil water storage, and B) urease, C) invertase and D) catalase activity in different soil layers plotted against one-time bentonite-humic acid (BHA) application rate in 2014 and 2015. The response of dependent variable (Y) to the BHA application rate (X) was fit individually to a piecewise linear plus plateau model (dotted lines), where the slopes of the regression lines were significant ( $P < 0.05$ ) for all parameters. Colors indicate different soil depths in all panels: 0-10 cm (red plus), 10-20cm (green ×), 20-40 cm (blue asterisk), and 40-60 cm (black circle).

**Fig. 2** Means of A) soil electrical conductivity, B) pH and C) bulk density (SBD) measured at crop harvest in 2014 and 2015 in the 0-60 cm soil profile plotted against one-time bentonite-humic acid (BHA) application rate. The response of dependent variable (Y) to the BHA application rate (X) was fit individually to a piecewise linear plus plateau model (dotted lines), where the slopes of the regression lines were significant ( $P < 0.05$ ) for all parameters, except 0-10 cm and 20-60 cm for soil bulk density in 2014 which was not significant. Colors indicate different soil depths in all panels: 0-10 cm (red plus), 10-20 cm (green ×), 20-40 cm (blue asterisk), and 40-60 cm (black circle).

**Fig. 3** Means of A) soil alkaline nitrogen (AN), B) available phosphorus (AP), C) available potassium (AK), and D) organic matter measured at crop harvest in 2014 and 2015 in the 0-60 cm soil profile plotted against one-time bentonite-humic acid (BHA) application rate. The response of dependent variable (Y) to the BHA application rate (X) was fit individually to a piecewise linear plus plateau model (dotted lines), where the slopes of the regression lines were significant ( $P < 0.05$ ) for all parameters, except 40-60 cm for AK in 2014 which was not significant. Colors indicate different soil depths in all panels: 0-10 cm (red plus), 10-20cm (green ×), 20-40 cm (blue asterisk), and 40-60 cm (black circle).

**Fig. 4** Relationships between the one-time bentonite-humic acid (BHA) application and A) grain yield (GY), B) total grain protein yield (TGP), C) evapotranspiration (ET), D) water use efficiency (WUE), E) partial factor productivity of nitrogen (PFPN) and F) biomass yield (BY) in 2014 and 2015. The response of dependent variable (Y) to the BHA application rate (X) was fit individually to a piecewise linear plus plateau model (dotted lines), where the slopes of the regression lines were significant ( $P < 0.05$ ) for all parameters.

**Fig. 5** Redundancy analysis (RDA) of the associations of crop performance measurements (total grain protein, grain and biomass yield) with soil biological, physio-chemical properties at a depth of 10–20 cm in 2014 and 2015. BHA 0, BHA 6, BHA 12, BHA 18, BHA 24 and BHA 30 represent none, 6, 12, 18, 24 and 30 Mg ha<sup>-1</sup> one-time application of bentonite-humic acid (BHA) treatment, respectively. SBD, soil bulk density; SEC, soil electrical conductivity; SWS, soil water storage; Ure, urease; Invert, invertase; Catal, catalase; AN, alkaline nitrogen; AP, available phosphorus; BY, biomass yield; TGP, total grain protein; GY, grain yield. The length of the arrow indicates the relative variance in the RDA axis explained by that factor; the angle between two arrows for two variables represents the correlation between these two variables.