



## Long-term biosolids land application influences soil health

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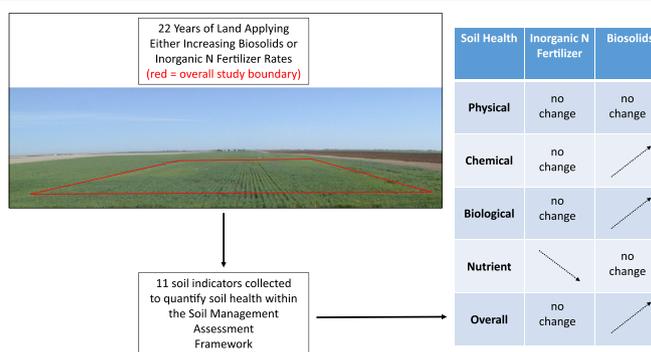
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### HIGHLIGHTS

- Overall soil health effects of biosolids land application have not been addressed.
- A 22-year biosolids research site and SMAF were used to address this knowledge gap.
- Biosolids increased soil chemical and biological health indices.
- As compared to inorganic fertilizers, biosolids improved soil biological health.
- Results support biosolids playing a pivotal role in agroecosystem sustainability.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Soil health assessments associated with organic amendment applications have primarily focused attention on manure or composts. Yet, quantifying specific changes in soil health associated with biosolids land applications has yet to be determined. Our objectives were to evaluate the changes in various soil indicators, and utilizing the Soil Management Assessment Framework (SMAF), quantify changes in soil indicator scores and soil health indices as affected by either increasing inorganic N fertilizer (0 up to 112 kg N ha<sup>-1</sup>) or biosolids (0 up to 11.2 dry Mg ha<sup>-1</sup>) applied every other year over 22 years. Soils were sampled (0 to 20 cm depth) following 22 years of N fertilizer or biosolids inputs to a dryland wheat-fallow (*Triticum aestivum* L.) rotation, 11 soil health indicators were monitored under SMAF guidelines, and indicators, indicator scores, and soil health indices were analyzed statistically. In general, increasing N fertilizer application rates had little effect on soil indicators, SMAF indicator scores or soil health indices. Increasing biosolids application rates increased soil organic C (SOC) and potentially mineralizable N (PMN). The SMAF indicator scores showed upward trends for soil pH, SOC, PMN, and microbial biomass C (MBC) associated with increasing biosolids application rates; discussing trends are important as these indicator scores are combined to provide soil health indices. Indeed, increasing biosolids application rates increased soil chemical and biological health indices, leading to an improvement in the overall soil health index. When comparing the overall N fertilizer to biosolids effect, biosolids applications significantly improved the soil biological health index. Results indicate that long-term biosolids land application to semi-arid, dryland wheat fallow rotations, similar to those studied, improve various aspects of soil health. These findings suggest that biosolids may play a pivotal role in dryland agroecosystem sustainability.

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### 1. Introduction

Biosolids land application, at agronomic rates, have been proven to meet crop nutrient demands (Barbarick and Ippolito, 2007) while maintaining crop yields similar to inorganic fertilizer inputs (Barbarick et al.,

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2012). However, despite widespread practice within the U.S., agronomic biosolids use remains controversial (Shober et al., 2003; US Environmental Protection Agency, 2018). When biosolids are land applied, concerns over losses or accumulation of soil elemental constituents (e.g., N, P, trace elements; Ippolito et al., 2014, 2007; Shober et al., 2003), changes in soil physical status (e.g., bulk density, aggregate stability; Kim et al., 2004; García-Orenes et al., 2005), or alterations in soil microbial community functionality (Brown et al., 2005; Sullivan et al., 2006a, 2006b) may be realized. Thus, previous research has focused attention on proving that agronomic biosolids land application can improve soil chemical, physical, and microbiological properties, but without a specific focus on soil health evaluations. The combined focus on soil chemical, physical, and microbiological properties is, in essence, the principle of soil health.

Soil health can be defined as the ability of soil to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and well-being (Karlen et al., 1997). Typically, soil health is quantified with respect to alterations in soil physical, chemical, nutrient, and biological attributes. Within the context of quantifying soil health in ecosystems receiving organic amendments, most often manured systems are studied. For example, Karlen et al. (2014) evaluated soil health in five Midwestern U.S. watersheds as affected by manure application. Surface soil (0 to 5 cm) health tended to decrease with manure application due to decreases in water stable aggregates, increases in bulk density, and slight alterations in soil organic C. Stott et al. (2012) identified soil health alterations following five decades of various agricultural practices, including continuously cropped or bermudagrass (*Cynodon dactylon*) with or without turkey (*Meleagris gallopavo* f. domestica) litter additions. The authors observed that: bermudagrass + turkey litter amended fields had the healthiest physical soil attributes; all management practices showed similar soil chemical attributes; the continuously cropped, continuously cropped + turkey litter, and the bermudagrass + turkey litter treatments had the healthiest soil nutrient attributes; and bermudagrass + turkey litter amended fields typically had the healthiest soil biological attributes. Recently, the Soil Health Institute published an introduction to their North American project to quantify soil health (Norris et al., 2020). Members of the Soil Health Institute research team have shown that including organic amendments in agroecosystems: significantly increased the active soil C by ~12% on average (Liptzin, 2020); increasing soil organic C tended to increase microbial biomass (Norris, 2020); trended towards increasing total soil N, water-extractable organic N, and nitrogen cycling enzymatic activity, while only utilizing synthetic inputs reduced total soil N (Cappellazzi, 2020); and aggregate stability increased by 6 and 8% when determined by wet sieving or a wet soil stability index method, respectively (Greub, 2020). However, bulk density, saturated hydraulic conductivity, or available water holding capacity did not change due to organic amendment inputs (Bean, 2020).

Although the Soil Health Institute has yet to create a framework by which overall soil physical, chemical, and biological attributes are combined to quantify soil health, the above findings suggest that, overall, positive changes occur when organic amendments are added to agroecosystems. However, the research by Karlen et al. (2014) and Stott et al. (2012) utilized a tool called the Soil Management Assessment Framework (SMAF; Andrews et al., 2004) to quantify soil physical, chemical, nutrient, biological, and overall soil health alterations with manure-amended agroecosystems; what is lacking in the literature is a quantification of soil health with respect to biosolids.

There are multitudes of studies that have quantified soil physical, chemical, nutrient, and biological alterations due to biosolids land application (e.g., Ippolito et al., 2014, 2007; Sullivan et al., 2006a, 2006b; Brown et al., 2005; García-Orenes et al., 2005; Kim et al., 2004; Shober et al., 2003), yet to our knowledge no studies have used the SMAF (or similar soil health quantification tools) to assess soil health under biosolids land application. Thus, the objectives of this study were twofold: first, to utilize a long-term biosolids land application site to quantify

alterations in soil physical, chemical, nutrient, and biological attributes; and second, to assess overall soil health, as compared to inorganic fertilizer applications, utilizing the SMAF as a means for quantification.

## 2. Materials and methods

### 2.1. Study location and sample collection

The study site was established in 1993, approximately 20 km north of Bennett, Colorado, USA on a fine, smectitic, mesic Aridic Argiustoll (Weld series; California Soil Resource Lab, 2008). Following ~60 d of air-drying, anaerobically digested, South Platte Renew Water Facility (Englewood, Colorado) biosolids were hand-applied at 0, 2.24, 4.48, 6.72, 8.96, and 11.2 dry Mg ha<sup>-1</sup> to 1.8 by 17.1 m plots. Biosolids applications comprised half the study, while the other half of the study included six urea fertilizer rates equal to 0, 22.4, 44.8, 67.2, 89.6, and 112 kg N ha<sup>-1</sup>. Nitrogen in the increasing biosolids application rates approximately equaled increasing urea-N application rates (Barbarick and Ippolito, 2000, 2007). Plots were arranged as a randomized complete block design with four replicates. Following application, all plots were tilled to a 20-cm depth with a rototiller. A winter-wheat fallow cropping rotation was followed, and thus biosolids or urea fertilizer was applied in August every other year from 1993 through 2015. Biosolids total N and P content from 1993 through 2013 can be found in Barbarick et al. (2017), while the 2015 biosolids contained 34,000 and 41,000 mg kg<sup>-1</sup> of total N and P, respectively.

Following wheat harvest in 2016, 40 bulk soil samples were obtained to a 20 cm depth within each plot using a 3.2 cm inner diameter soil probe. Soils were placed into a bucket, thoroughly mixed, and then placed in plastic sealable bag. The bags were immediately placed in coolers. An extra 20 cm deep soil core was obtained from each plot, placed in a metal can for gravimetric soil moisture content and bulk density (Bd) analyses.

All soils were returned to the lab, with soils in the metal cans weighed, oven-dried at 105 °C for 24 h, then weighed again for gravimetric moisture content and Bd determination. Bulk soils were immediately and entirely passed through an 8-mm sieve, with ~150 g of this field-moist soil placed in a sealable plastic bag and immediately stored at 4 °C for subsequent analyses. Approximately 150 g of the 8-mm sieved soil was passed through a 2-mm sieve and then air-dried, with the remainder of the 8-mm sieved soil also air-dried.

### 2.2. Soil health analyses

The Soil Management Assessment Framework (SMAF) is a soil health assessment tool developed jointly by the USDA-Agricultural Research Service and the USDA-Natural Resources Conservation Service (Andrews et al., 2004). The SMAF utilizes on-site soil taxonomy (including clay content; an overriding factor that can influence the other soil indicator data collected), climate, management, and in addition to clay content another 10 soil indicators to quantify soil health. Briefly, the SMAF utilizes the above information to classify soil physical [Bd, wet aggregate stability (WAS)], chemical [pH, electrical conductivity (EC)], nutrient [plant-available P and K], and biological [soil organic C (SOC), potentially mineralizable N (PMN), microbial biomass C (MBC), and β-glucosidase activity] soil health indices, and then calculates an overall soil health index. Unitless scores are assigned to each indicator based on previously tested scoring functions presented by Andrews et al. (2004; either more is better (e.g., SOC), less is better (e.g., Bd), or somewhere in the middle is better (e.g., plant-available P)), with weighted indices determined on a scale from zero to one based on the number of indicators within each soil health category above.

Briefly, soil clay content was determined using the hydrometer method (Ashworth et al., 2001) while soil Bd was determined as described above. Wet aggregate stability was determined using the 8-mm air-dried soil, a Yoder sieving instrument, following the method

outlined by Kemper and Rosenau (1986). Soil pH and EC were determined using the 2-mm air-dried soil and a 1:1 (soil:DI H<sub>2</sub>O) extraction after 2 h of shaking (Thomas, 1996; Rhoades, 1996). Plant-available P and K were determined using the 2-mm air-dried soil and an Olsen extraction (Olsen et al., 1954) and analyzed via inductively coupled plasma-optical emission spectroscopy. Potentially mineralizable N was determined using the 2-mm air-dried soil and a 28 d aerobic incubation outlined by Curtin and McCallum (2004), with mineralizable N (NH<sub>4</sub>-N + NO<sub>3</sub>-N) calculated as the difference between before and after the incubation period. β-glucosidase activity was determined on the 2-mm air-dried soil following the method by Green et al. (2007). Microbial biomass C was determined using the 8-mm field moist soil and the chloroform fumigation method by Allison (2008). Finally, a portion of the 2-mm air-dried soil was powder ground on a roller mill, and then used for determining total C via dry combustion (Nelson and Sommers, 1996) and inorganic C (Sherrod et al., 2002) analysis; SOC was calculated via difference.

Ten percent duplicates and blanks were utilized for the above analyses, with samples re-analyzed if duplicates were not within 5% error. It is also important to note that a specific soil standard was not utilized, as there currently is no standard soil available on the market that specifically targets soil health protocols.

### 2.3. Statistical analysis

Analysis of variance was performed on all soil indicators and soil health indices for increasing N fertilizer rate, increasing biosolids rate, and comparisons between N fertilizer and biosolids (independent of rate), using the Proc GLM model in SAS version 9.4 (SAS Institute, 2012) at a *p* < 0.10 (a significance level used in our previous publications; e.g., Barbarick et al., 2016, 2010, 1998; Barbarick and Ippolito, 2009). When significance was present, a Tukey adjusted pairwise comparison was used to identify effects of either increasing N fertilizer or biosolids application rates on soil indicators or soil health indices.

## 3. Results and discussion

### 3.1. Soil health indicators

Data pertaining to soil physical [Bd, WAS], chemical [pH, EC], nutrient [plant-available P and K], and biological [SOC, PMN, MBC, and β-glucosidase activity] soil health indicators are presented in Table 1. Increasing N fertilizer rates did not affect any soil characteristics, while increasing biosolids application rates did not affect the majority of indicators. A lack of change in the majority of indicators may have been due to tillage. In contrast, Nicholson et al. (2018) observed that aggregate stability increased by 33% after 20 years of repeated biosolids applications (2.9 to 3.4 Mg ha<sup>-1</sup> y<sup>-1</sup>) at a tilled site in England. The authors also found increased earthworm abundance when a low-metal containing biosolids was repeatedly applied over 20 years to several tilled sites in England.

However, increasing biosolids application rates caused a significant increase in SOC and PMN (Table 1). In a study of four locations within England, Nicholson et al. (2018) found that SOC and PMN increased by 10–17% and 19–51%, respectively, in soils receiving yearly biosolids applications (2.9 to 3.4 Mg ha<sup>-1</sup> y<sup>-1</sup>) over a 20 year period. Liptzin (2020) and Cappellazzi (2020) observed similar SOC and N trends in organically amended fields across North America. Cogger et al. (2013b) showed a similar SOC and N response in biosolids amended soils. Lehman et al. (2015) suggested that the single most important soil health indicator is soil organic matter, generally as measured by SOC. Increasing ecosystem SOC is intimately linked to positive changes in the soil microbial community, which is connected to enhanced nutrient cycling and retention (Lehman et al., 2015). Thus, there likely is a connection between increasing SOC and PMN within increasing biosolids application rates, as found by Sepúlveda-Varas et al. (2011) and supported by results from Cogger et al. (2013a). In support of this contention, increases in soil organic C content via biosolids application have been shown to increase bioactivity over time, enhancing nutrient

**Table 1**  
Mean Soil Management Assessment Framework physical [bulk density (Bd) and wet aggregate stability (WAS)], chemical [pH and electrical conductivity (EC)], nutrient [Olsen-extractable P and K], and biological [potentially mineralizable N (PMN), β-glucosidase activity, microbial biomass C (MBC), and soil organic C (SOC)] indicators as a function of increasing N (urea) fertilizer (kg ha<sup>-1</sup>) or biosolids (Mg ha<sup>-1</sup>) applications. Values inside parenthesis represent the standard error of the mean (n = 4). Different lowercase letters after an individual indicator and within either N fertilizer or biosolids indicates a significant difference as determined by a Tukey adjusted pairwise comparison.

|           | Physical indicators   |           | Chemical indicators |                       | Nutrient indicators    |                        | Biological indicators |                        |                        |  |
|-----------|-----------------------|-----------|---------------------|-----------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|--|
|           | Bd                    | WAS       | pH                  | EC                    | Olsen-P                | Olsen-K                | SOC                   | PMN                    | MBC                    | β-Glucosidase  |
|           | (g cm <sup>-3</sup> ) | (%)       |                     | (dS m <sup>-1</sup> ) | (mg kg <sup>-1</sup> ) | (mg kg <sup>-1</sup> ) | (%)                   | (mg kg <sup>-1</sup> ) | (mg kg <sup>-1</sup> ) | (mg pnp <sup>a</sup> kg <sup>-1</sup> soil h <sup>-1</sup> ) |
| N Fert.   |                       |           |                     |                       |                        |                        |                       |                        |                        |  |
| 0         | 1.28(0.06)            | 30.5(3.9) | 7.6(0.2)            | 0.26(0.05)            | 52.1(7.8)              | 646(46)                | 0.70(0.07)            | 15.7(2.1)              | 270(25)                | 29.4(2.8)  |
| 22.4      | 1.38(0.09)            | 37.6(4.9) | 7.7(0.2)            | 0.24(0.05)            | 26.4(7.7)              | 604(33)                | 0.67(0.08)            | 13.4(1.6)              | 221(14)                | 21.7(3.0)  |
| 44.8      | 1.30(0.04)            | 33.6(5.0) | 7.6(0.3)            | 0.22(0.05)            | 45.7(8.6)              | 651(35)                | 0.78(0.09)            | 15.5(1.1)              | 259(36)                | 24.8(3.0)  |
| 67.2      | 1.20(0.08)            | 35.1(2.9) | 7.6(0.2)            | 0.27(0.05)            | 63.1(13.5)             | 666(26)                | 0.71(0.06)            | 17.6(1.7)              | 274(38)                | 27.4(4.9)  |
| 89.6      | 1.37(0.08)            | 39.0(5.0) | 7.6(0.3)            | 0.16(0.05)            | 32.2(19.7)             | 639(81)                | 0.76(0.11)            | 16.4(2.2)              | 278(33)                | 28.1(4.8)  |
| 112       | 1.26(0.05)            | 33.2(3.9) | 7.7(0.2)            | 0.23(0.06)            | 27.5(6.1)              | 663(23)                | 0.72(0.04)            | 16.6(1.5)              | 247(28)                | 22.9(2.1)  |
| ANOVA     |                       |           |                     |                       |                        |                        |                       |                        |                        |  |
| p-Value   | 0.517                 | 0.119     | 0.500               | 0.352                 | 0.174                  | 0.881                  | 0.767                 | 0.342                  | 0.670                  | 0.566  |
| Biosolids |                       |           |                     |                       |                        |                        |                       |                        |                        |  |
| 0         | 1.27(0.06)            | 34.6(4.2) | 7.8(0.2)            | 0.18(0.04)            | 29.7(5.8)              | 542(60)                | 0.60(0.04)b           | 15.2(1.8)c             | 227(16)                | 22.6(3.0)  |
| 2.24      | 1.36(0.07)            | 33.0(3.5) | 7.8(0.2)            | 0.21(0.05)            | 43.7(6.4)              | 646(66)                | 0.76(0.08)a           | 17.0(1.5)bc            | 309(25)                | 23.9(4.0)  |
| 4.48      | 1.27(0.05)            | 32.8(4.5) | 7.7(0.2)            | 0.29(0.09)            | 72.0(13.8)             | 702(45)                | 0.85(0.14)a           | 21.2(2.9)ab            | 271(22)                | 31.3(1.1)  |
| 6.72      | 1.25(0.10)            | 34.0(2.3) | 7.7(0.2)            | 0.28(0.08)            | 68.7(25.4)             | 726(93)                | 0.85(0.08)a           | 18.1(1.2)bc            | 295(39)                | 34.4(4.2)  |
| 8.96      | 1.29(0.06)            | 34.0(3.6) | 7.6(0.3)            | 0.21(0.02)            | 49.6(12.5)             | 624(47)                | 0.85(0.12)a           | 22.3(2.6)ab            | 291(14)                | 25.6(3.1)  |
| 11.2      | 1.30(0.07)            | 38.5(2.7) | 7.5(0.3)            | 0.30(0.06)            | 70.6(14.9)             | 614(27)                | 0.90(0.10)a           | 25.3(2.4)a             | 292(30)                | 28.5(2.0)  |
| ANOVA     |                       |           |                     |                       |                        |                        |                       |                        |                        |  |
| p-Value   | 0.929                 | 0.552     | 0.935               | 0.281                 | 0.188                  | 0.138                  | <b>0.090</b>          | <b>0.038</b>           | 0.287                  | 0.164  |
| Overall   |                       |           |                     |                       |                        |                        |                       |                        |                        |  |
| N Fert.   | 1.30(0.03)            | 34.8(1.8) | 7.6(0.1)            | 0.23(0.02)            | 41.2(5.5)b             | 645(18)                | 0.72(0.03)b           | 15.9(0.7)b             | 258(13)                | 25.7(1.6)  |
| Biosolids | 1.29(0.03)            | 34.5(1.5) | 7.7(0.1)            | 0.24(0.03)            | 55.7(6.8)a             | 642(27)                | 0.80(0.05)a           | 19.8(1.2)a             | 281(12)                | 27.7(1.5)  |
| ANOVA     | 0.818                 | 0.796     | 0.114               | 0.510                 | <b>0.062</b>           | 0.916                  | <b>0.059</b>          | <b>&lt;0.001</b>       | 0.165                  | 0.324  |
| p-Value   |                       |           |                     |                       |                        |                        |                       |                        |                        |  |

Italicized, bold ANOVA p-values indicate significance at a *p* < 0.10.

<sup>a</sup> pnp = p-nitrophenol.

turnover and availability (Tian et al., 2008). Specific to the current study, Fierer et al. (2020) suggested that microbial measurements, such as N mineralization, might indicate a potential “hot spot” of biogeochemical processes (i.e., improvements in nutrient, and specifically in this case, N turnover within biosolids amended soils).

As compared to N fertilizer, biosolids increased Olsen-extractable P, SOC, and PMN; identical responses were observed by Badzmierowski et al. (2020), Yucel et al. (2015), and Heathwaite et al. (2006). Increases in Olsen-extractable P following biosolids application might be expected as biosolids contain appreciable quantities of P; increasing soil extractable P concentrations following biosolids application has been observed by numerous researchers (Yucel et al., 2015; Sepúlveda-Varas et al., 2011; Ippolito et al., 2007; Chambers et al., 2003; Shober et al., 2003). Based on the SMAF scoring function which follows a “somewhere in the middle is best” approach for extractable-P concentrations, this would fall within the middle portion of that scoring function curve. Yet, the SMAF index calibrations are not always in agreement with local regulatory guidelines. For example, many states within the U.S. and several European countries have P risk indices in place for environmental protection purposes (Buczko and Kuchenbuch, 2007). Since the current project occurred in Colorado, based on the Colorado P Risk Index (NRCS, 2012), Olsen-P concentrations would be considered a medium-high environmental risk category if between 40 and 80 mg kg<sup>-1</sup>. At these concentrations, considerations should be given to the avoidance of surface water contamination with elevated P concentrations, as suggested by Pepper et al. (2008). Fortunately, the Colorado P Risk Index also considers other factors that would reduce environmental P risk (e.g., dryland cropping practices, wind erosion factors, application method and timing, distance from water bodies, and other best management practices). In the future, the SMAF extractable-P scoring functions may need to be altered to account for P risk losses using specific state guidelines.

As compared to N fertilizer, biosolids increased SOC by ~11%. Chambers et al. (2003) observed relatively similar increases in SOC (8–23% increase in soil organic matter; equivalent to 5–13% SOC) in five European systems receiving increasing biosolids application rates (0 up to 96 Mg ha<sup>-1</sup>; applications over four to ten year time spans) as

compared to inorganic fertilizer. Cogger et al. (2013a, 2013b) and Spargo et al. (2008) observed similar SOC responses when biosolids were land-applied in no-till cropping systems. It is important to note that cumulative biosolids applications in the current study ranged from 0 to 134 Mg ha<sup>-1</sup>, similar to Chambers et al. (2003). Chambers et al. (2003) also noted increases in some soil physical characteristics associated with biosolids application, unlike in the current study.

The increase in PMN with biosolids, as compared to N fertilizer, suggests that biosolids contains N forms that may be mineralized throughout a growing season and thus could provide N needs to crops during critical grain-filling periods. Previous findings from our research team showed that agronomic biosolids application rates to dryland wheat-fallow agroecosystems resulted in 54% of the biosolids applied N remaining as residual soil N, with first year potentially mineralizable N available at 21 to 33% of the biosolids N applied (Barbarick and Ippolito, 2007; Barbarick et al., 1996). Chambers et al. (2003) also noted increases in total soil N, which in the long-term could increase plant-available N supply via mineralization of soil organic N reserves. Therefore, biosolids use could not only be an advantage to producers to supply N at critical growth stage periods via one pre-season application, but in conjunction with the increase in SOC may provide evidence that biosolids may improve soil health as compared to inorganic fertilizer application.

### 3.2. Soil health indicator scores

Data pertaining to soil physical, chemical, nutrient, and biological soil health indicator scores are presented in Table 2. The 22.4 and 89.6 kg ha<sup>-1</sup> N fertilizer application rates had lower Olsen-extractable P scores as compared to the other N fertilizer treatments, likely due to the larger variability associated with these two treatments causing the indicator score to tail to the left of the “somewhere in the middle is better” Olsen-P scoring function in SMAF. Increasing biosolids application rate did not affect any soil physical, chemical, nutrient, or biological indicator scores. However, as compared to N fertilizer, biosolids increased the SOC indicator score, related to the overall increase in SOC in biosolids as compared to N fertilizer treated plots (Table 1). This finding

**Table 2**  
Mean Soil Management Assessment Framework physical [bulk density (Bd) and wet aggregate stability (WAS)], chemical [pH and electrical conductivity (EC)], nutrient [Olsen-extractable P and K], and biological [potentially mineralizable N (PMN), β-glucosidase activity, microbial biomass C (MBC), and soil organic C (SOC)] indicator scores (0.00 to 1.00) as a function of increasing N (urea) fertilizer (kg ha<sup>-1</sup>) or biosolids (Mg ha<sup>-1</sup>) applications. Values inside parenthesis represent the standard error of the mean (n = 4). Different lowercase letters after an individual indicator and within either N fertilizer or biosolids indicates a significant difference as determined by a Tukey adjusted pairwise comparison.

|                  | Physical indicator scores |            | Chemical indicator scores |            | Nutrient indicator scores |            | Biological indicator scores |            |            |               |
|------------------|---------------------------|------------|---------------------------|------------|---------------------------|------------|-----------------------------|------------|------------|---------------|
|                  | Bd                        | WAS        | pH                        | EC         | Olsen-P                   | Olsen-K    | SOC                         | PMN        | MBC        | β-Glucosidase |
| <b>N Fert.</b>   |                           |            |                           |            |                           |            |                             |            |            |               |
| 0                | 0.93(0.06)                | 0.74(0.06) | 0.24(0.09)                | 1.00(0.00) | 1.00(0.00)a               | 1.00(0.00) | 0.06(0.01)                  | 0.96(0.03) | 0.70(0.07) | 0.03(0.00)    |
| 22.4             | 0.75(0.14)                | 0.84(0.07) | 0.22(0.09)                | 1.00(0.00) | 0.95(0.03)ab              | 1.00(0.00) | 0.06(0.01)                  | 0.90(0.05) | 0.53(0.06) | 0.03(0.00)    |
| 44.8             | 0.94(0.04)                | 0.78(0.09) | 0.29(0.13)                | 1.00(0.00) | 1.00(0.00)a               | 1.00(0.00) | 0.07(0.01)                  | 0.98(0.02) | 0.65(0.13) | 0.03(0.00)    |
| 67.2             | 0.93(0.06)                | 0.82(0.04) | 0.24(0.09)                | 1.00(0.00) | 1.00(0.00)a               | 1.00(0.00) | 0.07(0.01)                  | 0.99(0.01) | 0.69(0.10) | 0.03(0.00)    |
| 89.6             | 0.79(0.09)                | 0.86(0.06) | 0.31(0.18)                | 1.00(0.00) | 0.81(0.11)ab              | 1.00(0.00) | 0.07(0.01)                  | 0.98(0.01) | 0.71(0.10) | 0.03(0.00)    |
| 112              | 0.95(0.04)                | 0.78(0.06) | 0.20(0.09)                | 1.00(0.00) | 1.00(0.00)a               | 1.00(0.00) | 0.07(0.00)                  | 0.98(0.01) | 0.62(0.11) | 0.03(0.00)    |
| ANOVA            |                           |            |                           |            |                           |            |                             |            |            |               |
| <i>p</i> -Value  | 0.455                     | 0.235      | 0.500                     | –          | <b>0.047</b>              | –          | 0.700                       | 0.175      | 0.708      | 0.562         |
| <b>Biosolids</b> |                           |            |                           |            |                           |            |                             |            |            |               |
| 0                | 0.95(0.02)                | 0.81(0.06) | 0.15(0.08)                | 1.00(0.00) | 0.97(0.03)                | 1.00(0.00) | 0.05(0.00)                  | 0.94(0.05) | 0.56(0.07) | 0.03(0.00)    |
| 2.24             | 0.82(0.10)                | 0.78(0.06) | 0.18(0.10)                | 1.00(0.00) | 1.00(0.00)                | 1.00(0.00) | 0.07(0.01)                  | 0.99(0.01) | 0.82(0.05) | 0.03(0.00)    |
| 4.48             | 0.94(0.04)                | 0.77(0.06) | 0.21(0.12)                | 1.00(0.00) | 0.96(0.04)                | 1.00(0.00) | 0.09(0.02)                  | 0.99(0.01) | 0.71(0.08) | 0.03(0.00)    |
| 6.72             | 0.89(0.08)                | 0.81(0.03) | 0.20(0.09)                | 1.00(0.00) | 0.84(0.16)                | 1.00(0.00) | 0.08(0.01)                  | 1.00(0.00) | 0.75(0.12) | 0.03(0.00)    |
| 8.96             | 0.93(0.05)                | 0.80(0.06) | 0.26(0.14)                | 1.00(0.00) | 1.00(0.00)                | 1.00(0.00) | 0.09(0.02)                  | 1.00(0.00) | 0.78(0.04) | 0.03(0.00)    |
| 11.2             | 0.91(0.05)                | 0.87(0.04) | 0.30(0.15)                | 1.00(0.00) | 0.97(0.03)                | 1.00(0.00) | 0.09(0.01)                  | 1.00(0.00) | 0.76(0.08) | 0.03(0.00)    |
| ANOVA            |                           |            |                           |            |                           |            |                             |            |            |               |
| <i>p</i> -Value  | 0.769                     | 0.494      | 0.943                     | –          | 0.589                     | –          | 0.199                       | 0.411      | 0.232      | 0.158         |
| <b>Overall</b>   |                           |            |                           |            |                           |            |                             |            |            |               |
| N Fert.          | 0.88(0.04)                | 0.80(0.03) | 0.25(0.05)                | 1.00(0.00) | 0.96(0.02)                | 1.00(0.00) | 0.07(0.00)b                 | 0.96(0.01) | 0.65(0.04) | 0.03(0.00)    |
| Biosolids        | 0.91(0.03)                | 0.81(0.02) | 0.22(0.05)                | 1.00(0.00) | 0.96(0.03)                | 1.00(0.00) | 0.08(0.01)a                 | 0.99(0.01) | 0.73(0.04) | 0.03(0.00)    |
| ANOVA            |                           |            |                           |            |                           |            |                             |            |            |               |
| <i>p</i> -Value  | 0.571                     | 0.936      | 0.149                     | –          | 0.940                     | –          | <b>0.046</b>                | 0.124      | 0.124      | 0.332         |

Italicized, bold ANOVA *p*-values indicate significance at a *p* < 0.10.

is likely indicative of relatively long-term biosolids inputs to till systems, similar to increases in soil C found by Cogger et al. (2013a) when biosolids were incorporated into dryland wheat-fallow agroecosystems over a 16-year period of time. However, when agroecosystems are treated with other organic amendments or management practices are altered, variable responses have occurred. For example, Stott et al. (2012) found decreases in the SOC indicator score with turkey litter applied to tilled row crops as compared to relatively undisturbed agroecosystems. Karlen et al. (2014) found no significant SOC indicator score differences between manured and un-manured fields, attributable to the variability in SOC across the midwestern US soils studied; a finding observed in similar studies (e.g., Johnson et al., 2014).

It is important to note several trends in the N fertilizer and biosolids indicator score data, as even though some indicator scores may be non-significant, when compiled to produce soil physical, chemical, nutrient, biological, and overall soil health scores, these final scores may become significant with the SMAF (described below). A somewhat upward and downward trend existed for the biological indicators of PMN and MBC, respectively, with increasing N fertilizer rate. An upward trend existed for the chemical indicator, pH, with increasing biosolids application rate. Also, an upward trend existed for the biological indicators of SOC, PMN, and MBC with increasing biosolids application rate. Yucel et al. (2015) found significant increases in SOC, total N,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and MBC following 25 years of biannual biosolids applications (total loading was  $91.6 \text{ Mg ha}^{-1}$ , which falls within the cumulative biosolids applied of 0, 27, 54, 81, 108, or  $134 \text{ Mg ha}^{-1}$  in the current study). Similar results

were reported by Sciubba et al. (2013) who studied biosolids applications to two different soils over a 98 d laboratory incubation.

### 3.3. Soil health scores

Soil physical, chemical, biological, nutrient, and overall soil health index scores associated with increasing N fertilizer or biosolids application rates, or between N fertilizer and biosolids, are shown in Fig. 1. Increasing N fertilizer rates had no effect on soil physical, chemical, biological, or overall soil health indices (Fig. 1A). However, a slight decrease in the nutrient soil health index score associated with the 22.4 and  $89.6 \text{ kg ha}^{-1}$  N fertilizer rates was observed, with this decrease due to a tailing-off effect associated with greater variation in the Olsen-P content, as explained above. Within the inorganic fertilizer treatments, the upward PMN and the downward MBC trends within indicator scores (Table 2) negated each other, leading to no change in the biological soil health score. Opposite, the  $11.2 \text{ Mg ha}^{-1}$  biosolids application rate was greater than the control, while lower biosolids application rates were similar to the control with respect to the chemical soil health index. With respect to the biological soil health index, all biosolids application rates were similar, yet greater than the control. The increases in the chemical and biological soil health indices led to an increase in the overall soil health index (Fig. 1B); a similar increase in the overall soil health index associated with manure applications across watersheds was observed by Karlen et al. (2014). Finally, when comparing N fertilizer to biosolids applications, biosolids increased the biological

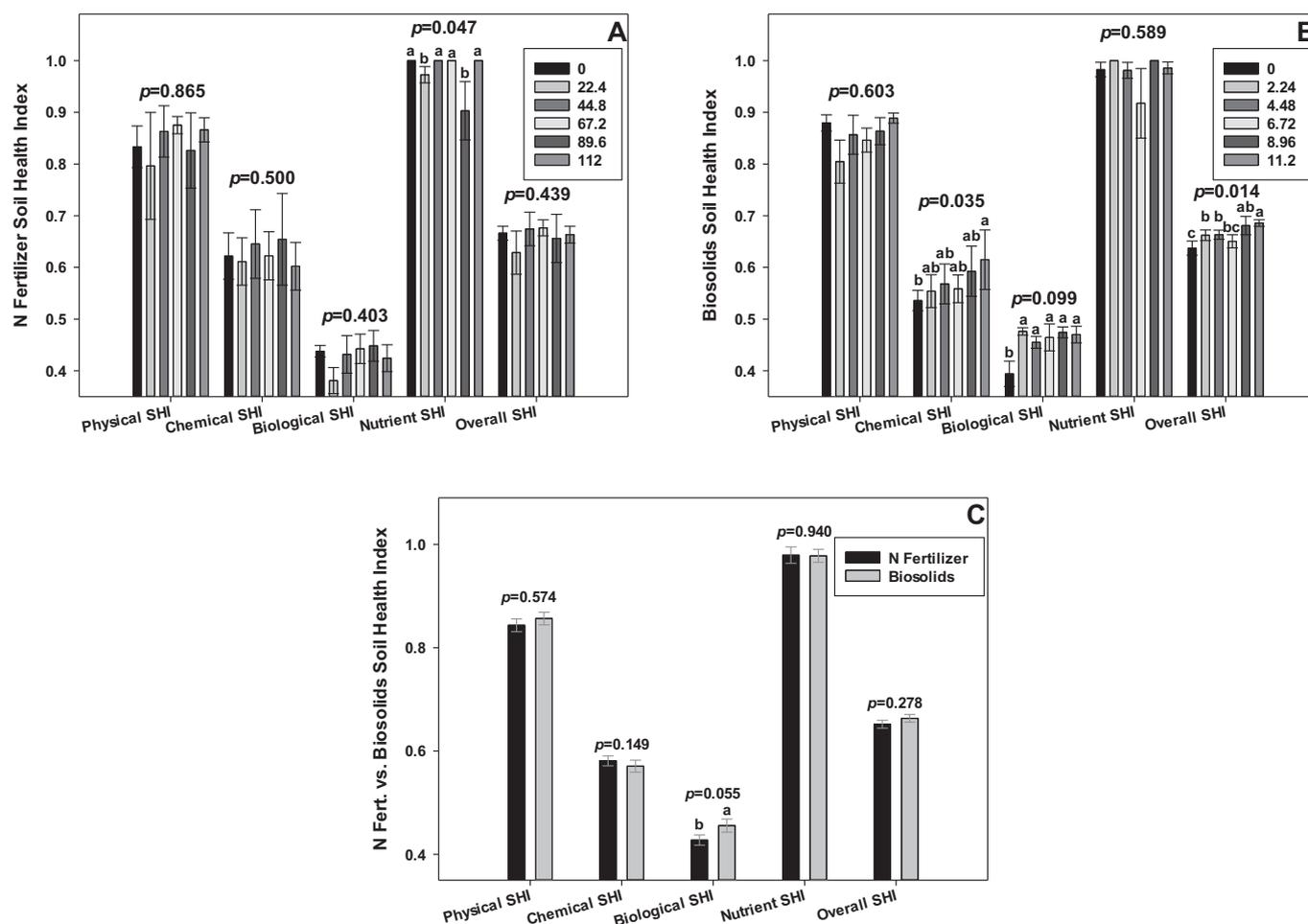


Fig. 1. Soil physical, chemical, biological, nutrient, and overall soil health changes due to increasing A) N fertilizer rate ( $\text{kg ha}^{-1}$ ) or B) biosolids application rate ( $\text{Mg ha}^{-1}$ ), and C) overall comparisons between N fertilizer and biosolids for all five soil health indices. Error bars represent one standard error of the mean.

soil health index as compared to N fertilizer (Fig. 1C). Given the relatively low biosolids application rates, it is interesting to note the significant difference within several soil health indices associated with increasing biosolids application rates, or between biosolids and N fertilizer for the biological soil health index. These differences are likely associated with 22 years of biennial biosolids land applications.

#### 4. Conclusions

The objectives of this study were to utilize the SMAF for evaluating potential alternations in soil physical, chemical, nutrient, and biological attributes, and overall soil health, within long-term (>20 yr), increasing biosolids as compared to inorganic fertilizer land application rates applied to a dryland wheat-fallow agroecosystem. Increasing N fertilizer rates caused minimal changes in soil characteristics, indicator scores, or soil health indices; a slight decrease in the soil nutrient health index, as quantified using the SMAF, was likely due to greater variability present in extractable soil P concentrations associated with several N fertilizer rates. In contrast, increasing biosolids application rates caused significant increases in SOC and PMN, suggesting that biosolids could provide for increased soil biogeochemical processes, as observed in the increase in soil extractable P, SOC, and PMN associated with biosolids applications as compared to N fertilizer applications. Although soil indicator scores were not significantly affected by increasing biosolids application rates, upward trends were present for soil pH, SOC, PMN, and MBC; SMAF indicator scores are combined to provide output associated with soil health indices, and thus observations in trends may be important. Indeed this held true, as: 1) increasing N fertilizer application rates only caused a slight decrease in nutrient soil health, associated with increased soil extractable P variability; 2) increasing biosolids application rates increased soil chemical and biological health indices, leading to an improvement in the overall soil health index; and 3) as compared to N fertilizer, biosolids led to an improvement in the biological soil health index. Findings indicate that long-term biosolids land application to semi-arid dryland cropping systems can improve soil health, suggesting that biosolids may play an important role in prolonged agroecosystem sustainability.

#### CRedit authorship contribution statement

All co-authors have contributed to the experiment design, data obtainment and text improvement, analysis and processing as well as manuscript editing, with all agreeing to submit to this journal.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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