

1 **Reanalysis Validates Soil Health Indicator Sensitivity and** 2 **Correlation with Long-term Crop Yields**

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13 14 **Keywords**

15 Soil health, yield, tillage, organic, CASH, North Carolina

16 17 **Core Ideas**

- 18 • Soil health metrics were sensitive in North Carolina soils
- 19 • Tillage intensity and fertility practices were especially differentiated by biological
20 soil health metrics
- 21 • Soil health metrics associated with labile organic matter correlated well with crop
22 yields

23

24 **Abstract**

25 Soil health (SH) refers to the ability of a soil to function and provide ecosystem
26 services. This study reanalyzes data from long-term agronomic management experiments
27 in North Carolina and addresses previous conclusions regarding the utility of SH test
28 metrics. Data for 15 SH indicators in the Comprehensive Assessment of Soil Health
29 framework from three long-term trials in North Carolina were analyzed to assess effects of
30 tillage intensity and organic vs. conventional management. This included four soil
31 biological indicators - organic matter (OM), active carbon (ActC), respiration (Resp) and
32 protein (Prot); four soil physical indicators - available water capacity (AWC), water stable
33 aggregation (Agstab), surface and subsurface penetration resistance (SurfHard, SubHard);
34 and seven soil chemical (fertility) indicators (P, K, Mg, Fe, Mn, Zn, pH). Corn (*Zea mays* L.)
35 and soybean (*Glycine max* L. Merr.) yield data and SH indicator values were correlated
36 using site-specific and multi-site datasets. Long-term management practices most
37 commonly showed significant impacts with AgStab (up to 2.2x), ActC (2.1x), Prot (2.3x),
38 and most chemical indicators. Tillage intensity had a greater impact than organic vs.
39 conventional management and linear regression of multi-year mean corn and soybean
40 response to tillage showed significant correlations with eight SH indicators, highest among
41 them ActC, Protein, Resp, and Mn ($R^2=0.85-0.93$). Contrary to previous conclusions, CASH
42 indicators, especially those related to labile C and N, responded well to management
43 practices and showed utility for soil health assessment in agronomic trials.

44

45 **Abbreviations:** ActC, active carbon, also known as permanganate oxidizable carbon, POXC;
46 AgStab, water stable aggregation; AWC, available water capacity; CASH, Comprehensive

47 Assessment of Soil Health, OM, organic matter; Resp, respiration during a 4-day incubation;
48 SurfHard, penetration resistance within the 0 to 15-cm depth range; SubHard, penetration
49 resistance within the 15 to 45-cm depth range; SH, soil health.

50

51 **Introduction**

52 Healthy well-functioning soils that enhance water and air quality, support human
53 health and habitation, and sustain plant and animal productivity are essential to ensuring a
54 sustainable future for an ever-growing global population (Karlen et al., 2003; Karlen and
55 Rice, 2015). Soil health (SH) refers to the ability of a soil to perform such functions based on
56 its inherent and dynamic characteristics (Karlen et al., 1997; Andrews et al., 2004; Idowu et
57 al., 2009). Therefore, within the context of land use and management goals, SH represents
58 an understanding of this resource as a dynamic, complex, and living system (Doran and Zeiss,
59 2000). The terms “soil quality” and “soil health” are used interchangeably in the literature
60 and can be considered equivalent (Bünemann et al., 2018), but within the past five years
61 stakeholder audiences and media sources have shown a preference for the latter term, which
62 we use herein.

63 The assessment of SH can be used as an indicator of sustainable land management
64 (Doran and Jones, 1996; Karlen et al., 1997). Traditional soil testing was and continues to
65 be essential, but it primarily focuses on soil chemical property measurements (*i.e.*, pH and
66 exchangeable or extractable nutrient concentrations) needed to evaluate soil fertility
67 (Moebius-Clune et al., 2016). Without question, traditional soil testing and plant analysis
68 have proven useful for increasing agricultural production, but the narrow focus on soil
69 chemical properties and processes has been regarded as a contributor to physical and

70 biological soil degradation (Tilman et al., 2002; Andrews and Carroll, 2001). The concept of
71 SH embraces a new comprehension of how soils function. It includes an understanding of
72 the physical, biological and chemical interactions that go well-beyond soil nutrient
73 quantities, and is needed to diagnose and quantify critical dynamic and inherent soil
74 properties and processes (Doran and Safley, 1997).

75 The Soil Management Assessment Framework (SMAF) was developed by Andrews et al.
76 (2004) as a comprehensive tool that is sensitive to textural class, sub-order soil organic
77 matter (SOM) content, Fe_2O_3 content, mineral class, climate, weathering class, slope,
78 sampling time, crop sequence, P analytical method and EC analytical method to evaluate
79 how land management practices impact soil functions (*i.e.*, physical, chemical, and
80 biological soil processes). Subsequently, the Comprehensive Assessment of Soil Health
81 (CASH) framework, initially referred to as the Cornell Soil Health Test (CSHT), was
82 developed based on the same paradigm and designed as a practical framework that directly
83 meets agricultural land manager and applied researcher needs. A CASH analysis
84 emphasizes identification of specific soil constraints within agroecosystems, thereby aiding
85 in the selection of land management solutions to increase productivity and minimize
86 environmental impact (Idowu et al., 2009). The utility of CASH indicators was initially
87 evaluated based on the relevance to soil functions/processes, sensitivity to land
88 management decisions, analytical cost, reproducibility of measurements, sampling
89 requirements, and potential to be estimated by statistical correlation or detected using
90 sensors (Moebius, 2007; Moebius-Clune et al., 2016).

91 The CASH framework was originally calibrated for soils within the northeastern USA,
92 but more recently has been applied to other geographic regions. A recent regional-scale

93 analysis of CASH showed significant SH differences between Midwestern, Northeastern and
94 Mid-Atlantic soils that were attributed in part to differences in farming systems (Fine et al.,
95 2017). Soil health studies using CASH have also been conducted at the landscape scale
96 (Moebius-Clune et al., 2011; Svoray et al., 2015;) and plot scale (Idowu et al., 2009;
97 Congreves et al., 2015; Kinoshita et al., 2017; Nunes et al., 2018), demonstrating that it can
98 effectively detect differences among agronomic management practices at multiple spatial
99 levels and with different types of soil. Similarly, the SMAF has been successfully used for SH
100 (soil quality) analysis in Brazil (Cherubin et al., 2016), Spain (Apesteguía et al., 2017), and
101 for many different soil management comparisons throughout the U.S. (e.g., Veum et al., 2015;
102 Hammac et al., 2016; Ippolito et al., 2017).

103 Roper et al. (2017) assessed the utility of CASH as well as the Haney Soil Health Test
104 (HSHT; Haney et al., 2006) and a standard test by the North Carolina Department of
105 Agriculture and Consumer Services (NCDACS; Hardy, 2014) using long-term experiments
106 in three physiographic regions of North Carolina. This included an evaluation of CASH's
107 ability to detect effects of long-term tillage practices and organic vs. conventional
108 management, as well as an analysis of correlations between soil health indicators and crop
109 yield. Regarding the latter, few studies have been able to quantify positive relations
110 between SH and crop yield, which is ostensibly a critical issue for farmer adoption.
111 Contrary to most previous studies using either CASH or the SMAF, the authors concluded
112 that SH indicators generally did not differentiate among agronomic management systems,
113 and moreover that SH scores did not show any correlation with crop yield. Those
114 conclusions have recently been cited by others (e.g., Rinot et al., 2019).

115 Questioning those conclusions based on multiple decades of experience in numerous
116 geographic regions, we decided to re-examine the data and determine whether the
117 research procedures, data analysis approach, or other factors negatively influenced the
118 conclusions. We concluded that the Roper et al. (2017) data clearly document management
119 effects on CASH indicators and demonstrate positive correlations between SH indicators
120 and yields of corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.), an elusive goal of past
121 studies. This suggests that, in line with recent studies (Congreves et al., 2015; Kinoshita et
122 al., 2017; Nunes et al., 2018), the use of CASH or SMAF indicators may in fact be quite
123 valuable for evaluating agronomic practices in programs such as those being coordinated
124 by the Soil Health Partnership (SHP) [[https://www.iowacorn.org/corn-](https://www.iowacorn.org/corn-production/environmental/soil-health-partnership)
125 [production/environmental/soil-health-partnership](https://www.iowacorn.org/corn-production/environmental/soil-health-partnership)], Soil Health Institute (SHI)
126 [<https://soilhealthinstitute.org/soil-health-research>], USDA-NRCS Soil Health Division
127 (SHD) [<https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/assessment/>],
128 and Foundation for Food and Agricultural Research (FFAR)
129 [<https://foundationfar.org/challenge/healthy-soils-thriving-farms/>]. Our goal therefore
130 was to perform a rigorous re-analysis of the above-mentioned NC dataset addressing
131 concerns with the research procedures and thereby determining: (i) effects of long-term
132 agronomic management practices on CASH indicators, (ii) relationships between CASH
133 indicators and crop yield for a range of tillage practices, and (iii) the overall utility of the
134 CASH framework for assessing agronomic management practices. We are not addressing
135 the results or conclusions related to the HSHT and NCDACS tests in the Roper et al. (2017)
136 study which have a narrower focus than either CASH and were not available in the
137 supplemental information.

138

139 **Materials and Methods**140 *Research Trials*

141 Data for this analysis were derived from Roper et al. (2017; Table S1 therein), which
142 contained the measured values of the CASH indicators for three long-term experiments
143 conducted within coastal plain, piedmont, and mountain physiographic regions of North
144 Carolina. These soil provinces have variable soil genesis and properties that are reflected in
145 inherent characteristics like texture and mineralogy, as well as distinct climate differences.
146 Corn and soybean yield data were derived from Table 7 (ibid). Site and management
147 history details are described in the original paper and only summarized herein:

148 The Goldsboro (coastal plain) research trial was conducted for 17 years on a site
149 where Wickham sandy loam (fine-loamy, mixed semiactive, thermic Typic Hapludults) was
150 the predominant soil with inclusions of Tarboro loamy sand (mixed, thermic Typic
151 Udipsamments). Agronomic treatments involved tillage practices and organic vs.
152 conventional nutrient and pesticide management. The study was initiated in 1999 (Mueller
153 et al., 2002) and included chemical no-till (NoTill) and conventional till (ConvTill) practices.
154 A 3-yr rotation, which since 2006 included corn, sorghum-sudangrass (*Sorghum ×*
155 *drummondii*), and double-crop soybean with winter wheat (*Triticum aestivum* L.) was
156 followed. The original experimental design also included two organic treatments, both
157 involving conventional tillage methods and adaptive cropping patterns. Since 2011,
158 ConvTill-Org1 involved a three-year rotation with corn, soybean and a 1-yr stale seedbed
159 with a sorghum-sudangrass cover crop. During the same time period ConvTill-Org2
160 involved a 3-yr rotation of corn, soybean, and sunflower (*Helianthus annuus* L.) with a rye

161 (*Secale cereal* L.) cover crop before soybean, and a rye and legume cover crop mixture before
162 corn and sunflower. The organic treatments utilized raw poultry litter as an external nutrient
163 source, while the conventional plots received an equivalent N rate using commercial fertilizer
164 sources. Yield measurements at this site were at times impacted by non-soil related factors,
165 notably extreme weed and insect pressures in the organic treatments and asynchronous crop-
166 years (Roper et al., 2017). They were therefore not considered for relating SH to crop yield.

167 The Reidsville (piedmont) research trial was conducted for 32 years on soil mapped as
168 Toast coarse sandy loam (fine, kaolinitic, mesic Typic Kanhapludults) and involved a
169 multitude of tillage treatments. It was initiated in 1984 with nine tillage treatments and
170 conventional chemical management that represented different levels of soil disturbance
171 ranging from minimal to severe (Cassel et al., 1995; Meijer et al., 2013). The multitude of
172 treatments and very subtle differences among some of them - coupled with high sampling
173 variability - challenged statistical analyses so the nine treatments were consolidated into
174 three groupings: (i) MinimumTill, combining no-till and in-row subsoiling in spring, (ii)
175 ChiselTill combining chisel plowing in spring, chisel plowing in fall, chisel plowing and
176 disking in spring, and chisel plowing and disking in fall, and (iii) MoldboardPlow, combining
177 spring and fall moldboard plowing and disking. This experiment involved multiple years of
178 crop yield measurements for both corn and soybean (Roper et al., 2017) that were used to
179 identify relationships with SH indicators.

180 The Mills River (mountain) research trial was conducted for 22 years on soil mapped
181 as Delanco silt loam (fine-loamy, mixed, semiactive, mesic Aquic Hapludults). It was
182 initiated in 1994 and designed as a 2x2 factorial with chisel and no-till practices being used
183 with conventional and organic management. An additional chisel plus disk tillage treatment

184 with no fertilizer or pesticide inputs was used as a control (Hoyt, 2005; 2007) but those
185 results were not used for this study. As with the Goldsboro site, yield measurements from
186 this experiment were impacted by non-soil related factors and complications related to crop
187 sequences (Roper et al., 2017), and therefore were not considered for SH correlations with
188 crop yield.

189

190 *Soil Sampling*

191 Soil samples were collected in late 2015 as discussed in Roper et al. (2017). Three sets of
192 penetrometer measurements (Field Scout SC-900, Spectrum, Aurora, IL) to a depth of 45
193 cm were collected from each plot when the soil moisture content was approximately at
194 field capacity. The highest resistance values within the 0-to-15-cm and 15-to-45-cm depths
195 were recorded as SurfHard and SubHard values. Three to five auger cores were collected to
196 a depth of 15 cm to obtain approximately 1400 cm³ of soil from each plot. Due to plot size
197 limitations, this sampling protocol deviated from recommended CASH procedures which
198 include more penetration measurements and a larger composite soil sample that is
199 subsequently mixed and subsampled (Moebius-Clune et al., 2016). This procedural
200 deviation likely increased sample variability and was the primary motivation to combine
201 treatments into more generalized groupings for statistical analysis, as discussed above.
202 After sampling, soil material was analyzed for multiple SH indicators at Cornell University
203 (Schindelbeck et al., 2016).

204

205 *Quantification of soil health indicators*

206 In addition to SurfHard and SubHard in-field measurements, a CASH analysis includes
207 measurements for two other soil physical indicators (Wet Aggregate Stability (AgStab),
208 Available Water Capacity (AWC); four biological indicators [organic matter (OM), active
209 carbon (ActC), autoclaved-citrate extractable protein (Protein), and soil respiration (Resp)]
210 as well as seven soil chemical property indicators (pH and extractable P, K, Mg, Fe, Mn, and
211 Zn). All analytical measurements were performed on disturbed, air-dried soil sieved to
212 pass a 2-mm screen. Appropriate corrections for sample water content after air-drying
213 were made after drying a subsample overnight at 105 °C.

214 Detailed laboratory procedures are available from Schindelbeck et al. (2016). In short,
215 AgStab was assessed using a rainfall simulator that generates 0.6 mm water drops and an
216 adjustable Mariotte-type tube to control hydraulic pressure (Ogden et al., 1997). A single
217 layer of aggregates was spread on a 0.25 mm mesh sieve that was placed 0.5 m below the
218 rainfall simulator to thus apply 2.5 J of energy over a 300-s period. AgStab was determined
219 as the fraction of soil remaining on the sieve after correcting for solid particles >0.25 mm
220 diameter.

221 Soil AWC was determined as the difference between water content at field capacity (θ_{fc})
222 and permanent wilting point (θ_{pwp}) based on a gravimetric analysis (g water g soil⁻¹).
223 Subsamples were saturated and equilibrated at -10 kPa (θ_{fc}) and -1500 kPa (θ_{pwp}) on
224 ceramic high-pressure plates (Soil Moisture Equipment Corp., Goleta, CA; Topp et al., 1993).

225 Soil OM content was determined by mass loss on ignition after two hours in a 500° C
226 muffle furnace. Active C was quantified by measuring absorbance with a handheld
227 spectrophotometer (Hach, Loveland, CO) after oxidizing duplicate, 2.5 g soil samples with 20

228 mL of 0.02 M potassium permanganate (KMnO_4) solution (pH 7.2). This measurement is also
229 referred to in the literature as permanganate oxidizable carbon, POXC.

230 Soil Resp was measured in duplicate after a 4-d incubation using a modified Haney and
231 Haney (2010) method where soil was placed in a glass jar with a KOH-based CO_2 trap. The
232 amount of CO_2 respired was determined by measuring the change in electrical conductivity
233 of the solution with an OrionTM DuraProbeTM 4-Electrode Conductivity Cell (ThermoFisher
234 Scientific, Inc., Waltham, MA). The necessary background correction for atmospheric CO_2
235 was quantified using blank (*i.e.*, no soil) incubations.

236 Protein content was measured by extracting a subsample with 0.02 M sodium citrate (pH
237 7), concentrating the sample through a series of centrifugation and autoclaving steps (Wright
238 and Upadhyaya, 1996), and then quantifying soil protein content using a bicinchoninic acid
239 assay with a bovine serum albumin standard curve.

240 Soil pH was measured in a 1:1 soil:water slurry. Plant available soil nutrient
241 concentrations (P, K, Mg, Fe, Mn and Zn) were measured using inductively coupled plasma
242 optical emission spectrometry (SPECTRO Analytical Instruments Inc., Mahwah, NJ) after
243 extracting with a Modified Morgan solution (ammonium acetate plus acetic acid, pH 4.8;
244 McIntosh, 1969). All nutrient contents were calculated per mass of soil (mg kg^{-1}).

245

246 *Data analysis*

247 Pearson product-moment correlation coefficients were computed for every pair of
248 soil health indicators to create correlation matrices for the pooled dataset that included each
249 individual trial, as well as all trials combined. Data for each trial were analyzed for significant
250 treatment effects using analysis of variance and means separation with the Tukey post-hoc

251 test for randomized complete block designs. Regression analysis was performed on corn and
252 soybean yields vs. SH indicators for the Reidsville (piedmont) experiment, which contained
253 16 years of continuous yield data (Roper et al., 2017). All statistical analyses and graphing
254 were performed using RStudio software version 1.0.143 (R Core Team, 2016).

255

256 **Results and Discussion**

257 *Site Comparisons and Correlation Analysis*

258 Soil health for each site was characterized by mean values (Table 1). The piedmont site
259 (Reidsville) generally showed the least favorable values for physical and biological
260 indicators, possibly because it only involved row crops and did not include any poultry litter
261 additions or cover crop. The coastal plain (Goldsboro) site showed lower OM, but not
262 commensurately lower Protein, ActC, and Resp, suggesting higher OM quality compared to
263 the piedmont and mountain sites. That response was quite likely associated with the organic
264 amendments and less organo-mineral bonds with coarser texture. The sites were strongly to
265 moderately acidic (pH 5.4-5.6) but generally showed adequate levels of crop nutrients based
266 on CASH interpretations (Moebius-Clune et al., 2016) , confirming that the sites generally
267 had good fertility management.

268 The SH indicators from each research site were compared with mean CASH database
269 values for coarse and medium textured soils in the Mid-Atlantic (Table 1). Those reference
270 data represent a diverse group of cropping systems and management practices within the
271 region (Fine et al., 2017). Mean AgStab values for the NC research sites were well below the
272 Mid-Atlantic equivalents for the same textural groups, and the biological indicators (OM,
273 Protein, Resp, and ActC) were also below the Mid-Atlantic average. Those results suggest

274 that the soils at the three research sites can be considered biologically and physically
275 degraded, which is consistent with the generally low crop productivity. For example, corn
276 grain yields averaged 6.21 and 4.89 Mg ha⁻¹ (99 and 78 bu ac⁻¹) for the coastal plain and
277 piedmont sites, which is well below the 3-yr (2013, 2015, and 2016) NC average of 8.03 Mg
278 ha⁻¹ (128 bu ac⁻¹; NASS, 2018). Soybean yields for the piedmont site averaged 2.94 Mg ha⁻¹
279 (44 bu ac⁻¹) which was higher than the state average for the same 3-yr period (2.40 Mg ha⁻¹
280 or 36 bu ac⁻¹), while fresh weight of sweetcorn averaged 12.7 Mg ha⁻¹ (1134 cwt ac⁻¹) at the
281 mountain site (Table 7 in Roper et al., 2017; Figs 1, 2). In addition to generally low crop
282 yields, interpretations in the previous analysis were based on CASH scores rather than
283 measured values (Roper et al., 2017). This could be problematic since CASH scores are based
284 on sigmoidal functions (Moebius-Clune et al., 2016) which show small rates of change at the
285 low end of the curve, thereby diminishing differences in measured values from agronomic
286 practices.

287 Pearson correlations among CASH indicators were developed for four cases: each of
288 the three trials individually, and all trials combined (Tables 2-5). When SH data from all trials
289 were pooled (n=64), half (53) of the 105 possible correlations among SH indicators were
290 significant at $\alpha=0.05$ and a third (37) were significant at $\alpha=0.01$. Significant correlations
291 were more or less equally found among physical, biological and chemical indicators, but the
292 highest r-values tended to involve biological indicators or Mg. Soil OM generally showed
293 only modest correlations with other biological indicators, suggesting some orthogonality
294 (*i.e.*, OM quantity and quality were somewhat independently expressed, e.g., the coastal plain
295 soils tended to have higher Protein and ActC contents relative to OM). Among these

296 indicators of labile organic matter, Protein, ActC, and Resp showed higher correlations, and
297 were better correlated with AgStab than OM.

298 Less significant correlations were observed for individual sites, presumably due to a
299 combination of less statistical power from a lower number of samples and smaller data
300 ranges within individual sites. Correlation patterns for each site (Tables 3-5) also differed
301 compared to the pooled data (Table 2), presumably reflecting treatment effects rather than
302 soil type differences. Notably, individual research sites showed high correlations of AWC
303 with OM, Mn, and Mg. Magnesium also correlated with other biological indicators and to a
304 lesser extent physical indicators. Otherwise, chemical indicators showed limited inter-
305 correlations. Overall, different correlations among SH indicator data from pooled and
306 individual sites suggest variable impacts for dynamic soil properties related to agronomic
307 management practices compared to inherent soil properties associated with the
308 geographically separated trial sites and soil types (Table 2).

309

310 *Treatment effects*

311 The three trials focused on different agronomic management practices, utilized different
312 crop sequences, and were conducted in different regions of the state with dissimilar soils, all
313 critical factors which were differentially expressed (Table 6). For example, treatment factors
314 were generally less significant ($\alpha=0.05$) at the Goldsboro (coastal plain) site than at the
315 Reidsville (piedmont) and Mills River (mountain) sites. Notably, the Reidsville site, which
316 only involved tillage comparisons, showed more significant treatment effects than either site
317 where different tillage practices were combined with conventional vs. organic management.

318 For all three sites, treatment effects were significantly expressed ($\alpha=0.10$) for ActC,
319 Agstab, and P, and at two of three sites significant treatments were measured for Protein,
320 pH, K, Mg, and Zn (Table 6). Other studies also found ActC and Agstab to be sensitive
321 indicators, especially compared to OM, which often shows small non-significant effects from
322 agronomic management practices (Idowu et al., 2009; Congreves et al., 2015; Kinoshita et al.,
323 2017; Nunes et al., 2018).

324 For the Goldsboro (coastal plain) trial, average AgStab was significantly higher ($\alpha=0.05$)
325 for No-Till than ConvTill-Org2, but for plant-available P NoTill was the lowest (Table 7). This
326 suggests that the organic system with poultry litter additions benefited from soil nutrient
327 additions, but had greater soil physical impediments due to tillage. ActC also showed the
328 highest numerical values for NoTill, but statistically they were not sufficient to be significant
329 in a means comparison ($\alpha=0.05$).

330 The Reidsville (piedmont) trial involved a range of tillage practices that were pooled
331 into three groups, MinimumTill, ChiselTill and MoldboardPlow (Table 7). For most biological
332 and physical SH indicators, tillage effects followed a consistent pattern of
333 MinimumTill>ChiselTill>MoldboardPlow, which were significant ($\alpha=0.05$) for Agstab,
334 Protein, Resp, and ActC. Tillage effects on chemical indicators were more variable, with only
335 Mg and Mn showing the same pattern. Intensive tillage accelerates decomposition of plant
336 biomass due to higher O₂ availability and exposure of older, physically-protected soil organic
337 carbon (Reicosky, 1997; Reicosky et al., 2011).

338 Tillage has thus been shown to increase CO₂ emissions (Melland et al., 2017), reduce
339 surface soil OM content (Kumar et al., 2017), and decrease the soil's ability to retain nutrients
340 and maintain its physical quality (Martínez et al., 2016; Alhameid et al., 2017). Our results

341 support the hypothesis that intensive tillage affects OM decomposition and impacts labile C
342 and N fractions (Protein and ActC) more than total OM. Also, the Resp indicator shows higher
343 decomposition rates for reduced tillage soil after it is disturbed by sample processing,
344 suggesting that the labile organic material is better retained when left undisturbed. Low OM
345 was also associated with decreased AgStab, an important indicator of physical soil quality.
346 In a study with NC and Virginia soils, Franzluebbbers (2018) and Franzluebbbers et al.
347 (2018a,b) conclude that the assessment of readily-decomposed C and N through the Resp
348 test is a better predictor of plant N availability than Total N and can be used to optimize
349 supplemental N fertilizer rates. Yost et al. (2018) also found that Resp explained some of the
350 variability in corn N response in eight Midwest states.

351 The Mills River (mountain) trial involved a factorial experiment that we analyzed to
352 contrast organic vs. conventional and NoTill vs. ChiselTill (Table 7). Organic treatments
353 focused on the use of cover crops and poultry litter, two practices that generally resulted in
354 more favorable physical and biological indicator values than conventional treatments, but
355 the only significant ($\alpha=0.05$) difference was for ActC and there were no significant
356 interaction effects. Many chemical indicators had higher values with organic management,
357 suggesting that effective nutrient additions through poultry litter likely exceeded those from
358 inorganic fertilizer. Except for surface penetration resistance (SurfHard), NoTill practices
359 consistently showed more favorable physical and biological SH indicator values than
360 ChiselTill, with significant differences ($\alpha=0.05$) for AggStab, Protein, P and Zn. The Mills
361 River experiment thus suggests that tillage and organic vs. conventional treatment effects
362 are differentially expressed through SH indicators.

363 This re-analysis of the NC data counters previous interpretations (Roper et al., 2017) and
364 is consistent with results from New York trials involving tillage practices, crop rotations, and
365 cover crop treatments that showed CASH indicators could differentiate among various
366 management practices (Nunes et al., 2018). In these trials, ActC, Protein, Resp, AgStab and
367 SurfHard showed significant responses to tillage (no-till, conventional till), and AWC to cover
368 cropping. Similarly, Congreves et al. (2015) measured significant responses of CASH
369 indicators (AgStab, pH and Zn) to tillage treatments (no-till, conventional till) in an Ontario
370 study. AgStab, P, and Mn also responded to crop rotation effects. Furthermore, an
371 assessment of a long-term tillage (plow, no-till) and crop residue management (removed or
372 retained) study by Kinoshita et al. (2017) showed that 40-year effects were discernable in
373 the 0-15 cm layer for all measured biological indicators and the majority of physical (notably
374 AgStab) and chemical indicators. Effects in some cases were also detected within the subsoil.
375 In ten European long-term experiments involving tillage and organic input management,
376 ActC (POXC) was determined to be the most sensitive and useful indicator for labile C
377 (Bünemann et al., 2018; Bongiorno et al., 2019). Collectively, these reports are in agreement
378 with our results showing that CASH indicators can differentiate agronomic management
379 effects.

380

381 *Soil Health and Yield*

382 Demonstrating positive relationships between soil health and crop yield is of great
383 interest to farmers as it could justify management investments in practices such as reduced
384 tillage, adding organic inputs, or altering rotations. But this has been difficult, especially in
385 experimental trials due to often inconsistent yield data or confounding impacts (e.g., pest

386 pressure, weather variability, and/or extraneous management factors). Roper et al., (2017;
387 Fig. 2 therein) used the NC dataset to determine relationships between overall soil health
388 scores and crop yield for the piedmont and mountain sites but found no correlation. We
389 hypothesize that this was primarily due to the use of overall soil health scores (masking
390 individual indicator effects) and confounding impacts associated with non-soil factors
391 (notably pest pressures). Nevertheless, the Reidsville (piedmont) site provided an excellent
392 experimental dataset to evaluate correlations between individual CASH indicators (rather
393 than scores) and yield, because (i) data were available from 17 corn harvests (between
394 1987 and 2015) and 10 soybean harvests (1990 to 2014), (ii) there was a gradient of tillage
395 intensities, and (iii) there were no apparent confounding factors affecting crop yields at
396 this site (as opposed to the coastal plain and mountain experiments).

397 Linear regression line plots showing relationships between various SH indicators and
398 yields of corn (Fig. 1) and soybean (Fig. 2) had high annual variability as expressed by
399 standard deviation (sd) bars (pooled values of 2.72 and 1.21 Mg ha⁻¹ for corn and soybean,
400 respectively). Corn yields were less than 7 Mg ha⁻¹ (112 bu ac⁻¹) with about half of the
401 means. Multi-year mean yield values, however, were often closely correlated with SH
402 indicators, especially for the biological properties and processes (Figs 1, 2). Eight soil
403 health indicators (Agstab, OM, Protein, Resp, ActC, P, Mg and Mn) showed significant
404 ($\alpha=0.10$) linear regression effects with mean corn and soybean yields (Table 8). This
405 implies that tillage-related SH differences as indicated by the SH values can on average be
406 expected to result in higher crop yields. For each SH indicator the linear relationship with
407 mean corn and soybean yield generally followed the pattern MinimumTill > ChiselTill >
408 MoldboardPlow, especially for the biological measurements (Figs. 1, 2). This implies that

409 reduced tillage resulted in better SH indicator values that in turn were associated with
410 higher average crop yields. This experiment thus provides some of the very best-available
411 results linking agronomic management practices to both soil health and yield benefits, and
412 counters conclusions by Roper et al. (2017) that SH indicators could not be correlated to
413 crop yield, which is relevant to commercial farmers.

414 For corn yields, the highest significant regression coefficients and associated R^2_{adj}
415 values (Table 8) followed the order of Protein>ActC>Mn>Resp>Aggstab>P>Mg>OM, while
416 for soybean yield they were ActC>Resp>Mg>Mn>Protein>P>Aggstab>OM (Figs 1, 2; note:
417 X-Y plots are ordered by R^2_{adj} value). Several insightful conclusions can be drawn from this
418 re-analysis:

- 419 1. Biological indicators associated with labile C and N show the strongest linear
420 regression fit with mean yield for both crops: ActC had very high R^2_{adj} values of 0.93
421 and 0.85 for mean soybean and corn yields, respectively, and Resp shows R^2_{adj}
422 values of 0.90 for mean soybean yield and 0.75 for corn. Protein values showed the
423 highest fit with mean corn yield ($R^2_{adj}=0.88$), but a lower correlation with soybean
424 yield (0.55), suggesting that a legume crop would benefit less from high soil Protein
425 levels -- and presumably the associated organic nitrogen -- than a non-legume crop.
426 This makes biological sense.
- 427 2. Soil OM levels showed relatively weak regression fits with mean yield (ranked 8th
428 for both corn and soybean; $p=0.06$ and 0.09 , respectively), while OM quality
429 indicators (*i.e.*, Protein, ActC) correlated much better, suggesting that OM quality
430 may be more relevant to crop yield than OM quantity.

- 431 3. Strong regression fits between crop yield and biological indicators suggest that the
432 negative impacts of intensive tillage on labile organic C fractions that are most
433 readily decomposed also adversely affect crop yield. Notably, Protein represents low
434 C:N organic matter that is readily used as a microbial food source and ActC mimics
435 organic matter decomposition including more recalcitrant forms (Weil et al, 2013;
436 Romero et al., 2018).
- 437 4. Mn is strongly impacted by tillage intensity, which in turn correlates well with mean
438 corn and soybean yield ($R^2_{adj}=0.85$ and 0.65 , respectively). Unlike other crop
439 nutrients, Mn was not managed through external applications and Mn contents for
440 this experiment were not out of line with regional averages (Table 1). Recent studies
441 have shown that Mn redox cycling is important in organic matter decomposition
442 (Keiluweit, 2015), which according to these results is impacted by tillage intensity.
- 443 5. AgStab is negatively impacted by tillage intensity and shows modest correlations
444 with yield, presumably due to aggregation effects from higher biological activity
445 (Magdoff and van Es, 2009), as evidenced by the biological SH indicators (Table 8).
- 446 6. Weaker correlations were observed for P and Mg (Fig. 1, 2), which presumably
447 relates to their enhanced availability with higher organic matter quality.

448

449 **Conclusions**

450 This study re-analyzed data from three long-term agronomic experiments in North
451 Carolina and conveys different perspectives from the paper by Roper et al. (2017), which
452 had concluded that the CASH framework and two other soil tests have limited ability to
453 discern among management practices. It had also concluded that there was a lack of

454 correlation between SH measurements and crop yield. Our analysis utilizes more nuanced
455 interpretations and is mostly in disagreement with those conclusions, but corroborates
456 other previous research on the utility of SH indicators. This is in part due to the fact that
457 the Roper et al. (2017) analysis was negatively impacted by low statistical power from high
458 sample variability, interpretations based on non-linear scoring functions that obscured
459 effects of individual treatments, as well as insufficient differentiation of the performance of
460 CASH from other soil tests (*i.e.*, NSHT and NCDACS). Moreover, their inference that overall
461 SH scores and crop yields were not correlated was strongly confounded by several non-soil
462 factors (*i.e.*, very low crop yields in some years, and pest pressures related to organic
463 practices). Furthermore, the analyses considered SH scores rather than individual indicator
464 values, which was problematic due to the quality of the soils.

465 Our analysis provides a different perspective and concludes that multi-functional
466 soil health indicators (biological, physical, chemical) indeed offer valuable insights for
467 interpreting long-term effects of agronomic management practices. Notably, we
468 demonstrated that different management practices variably impact different aspects of SH,
469 especially indicators associated with labile organic matter (ActC, Protein, Resp, AgStab).
470 Changes in tillage intensity appear to have greater impacts than organic vs. conventional
471 practices. Also, correlations among SH indicators varied based on the geographic scope of
472 the analysis and whether it involved a single-location trial or multiple trials.

473 Furthermore, the piedmont trial involving a range of tillage intensities and 16-year
474 cropping data offered unique insights into correlations between SH indicators and crop
475 yields as impacted by tillage. Although annual variability of corn and soybean yields was
476 high, the long-term average yields showed very good linear regression fits with SH

477 indicators related to organic matter quality. This suggests that labile sources of C and N are
478 important to soil health and crop performance. Results also show that Mn, which plays a
479 role in organic matter dynamics, is impacted by tillage practices and in turn correlates with
480 yields.

481 Overall we conclude that, contrary to previous inferences from these trials, (i)
482 comprehensive soil health assessment through the CASH framework was able to discern
483 effects of agronomic management practices (tillage, organic practices), (ii) biological
484 indicators associated with labile C and N are most impacted by management practices,
485 especially tillage, and (iii) SH indicators can be related to yield of corn and soybean under
486 varying tillage intensities, but scoring curves for soil health may need to be regionalized.

487

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491

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671 variability.

672 Table 1. Means of CASH soil health indicators for the coastal plain, piedmont and mountain sites, as well as the CASH database for Mid-Atlantic
 673 soils (from Fine et al., 2017).

Site	region	n	texture	AWC [†] m ³ /m ³	SurfHard ----- MPa -----	SubHard	Agstab %	OM %	Protein mg/g	Resp mgCO ₂ /g	ActC mg/kg	pH	P ----- mg/kg-----	K	Mg	Fe	Mn	Zn
Goldsboro	coastal plain	12	SL / LS	0.16	1.53	2.75	11	1.7	4.5	0.35	320	5.4	11.7	122	93	4.9	7.4	1.9
Reidsville	piedmont	32	SL	0.15	1.82	3.59	9	2.5	3.1	0.34	289	5.6	8.8	100	126	3.0	4.4	1.9
Mill River	mountain	20	SiL	0.21	1.48	2.61	12	2.6	4.0	0.39	312	5.6	6.3	123	151	4.2	6.6	0.8
database	Mid- Atlantic	101	coarse	0.11	1.25	2.21	45	2.2	6.4	0.52	335	6.0	14.2	92.5	87	3.5	7.4	1.9
database	Mid- Atlantic	317	medium	0.22	1.34	2.00	43	4.1	10.0	0.86	564	6.2	23.2	164	173	4.7	17.2	2.0

[†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard, penetration resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic matter; Resp, respiration during a 4 day incubation; ActC, active carbon.

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675

676 Table 2. Pearson correlations among CASH soil health indicators for all coastal plain, piedmont and
 677 mountain samples with $p < 0.05$ (bolded numbers $p < 0.01$; $n = 64$).

	AWC [†]	Surf Hard	Sub Hard	Ag Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
AWC															
SurfHard															
SubHard	-0.59	0.37													
AgStab	0.28		-0.28												
OM	0.47			0.25											
Protein	0.35		-0.55	0.55											
Resp	0.31	-0.30	-0.34	0.28	0.41	0.48									
ActC	0.25		-0.30	0.56	0.39	0.67	0.73								
pH							0.59	0.37							
P	-0.37				-0.37	0.50	0.27	0.37							
K	0.38	-0.28	-0.34		0.29	0.31	0.52	0.47	0.36						
Mg	0.53		-0.26	0.30	0.78		0.64	0.59	0.52		0.60				
Fe									-0.24	-0.36			-0.41		
Mn	0.57		-0.52	0.25		0.43	0.30	0.24			0.35				
Zn	-0.41									0.42		-0.27			

678 [†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard,
 679 penetration resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic matter; Resp,
 680 respiration during a 4-day incubation; ActC, active carbon.

681

682 Table 3. Pearson correlations among CASH soil health indicators for coastal plain (Goldsboro) samples
 683 with $p < 0.05$ (bolded numbers $p < 0.01$; $n = 12$).

	AWC [†]	Surf Hard	Sub Hard	Ag Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
AWC															
SurfHard															
SubHard	-0.61	0.65													
AgStab		0.61													
OM	0.87	-0.57	-0.62												
Protein															
Resp	0.67	-0.59			0.77										
ActC						0.61									
pH	-0.63						0.70								
P															
K		-0.63		-0.79	0.75		0.81		0.70						
Mg	0.76	-0.59	-0.62		0.93		0.79		0.62		0.79				
Fe															
Mn	0.84				0.66						-0.64				
Zn										0.70					

684 [†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard, penetration
 685 resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic matter; Resp, respiration
 686 during a 4-day incubation; ActC, active carbon.

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689 Table 4. Pearson correlations among CASH soil health indicators for all piedmont (Reidsville) samples
 690 with $p < 0.05$ (bolded numbers $p < 0.01$; $n = 32$).

	AWC [†]	Surf Hard	Sub Hard	Ag Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
AWC															
SurfHard															
SubHard															
AgStab															
OM	0.68			0.59											
Protein			-0.44	0.47											
Resp				0.47	0.37	0.65									
ActC	0.40			0.67	0.58	0.73	0.84								
pH							0.60	0.40							
P						0.67	0.50	0.40							
K							0.49	0.46	0.47						
Mg	0.41			0.69	0.75	0.40	0.69	0.80	0.47		0.55				
Fe					-0.51					0.38		-0.52			
Mn				0.53	0.49	0.47	0.42	0.51							
Zn			-0.35								-0.40			0.59	

691 [†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard, penetration
 692 resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic matter; Resp, respiration
 693 during a 4-day incubation; ActC, active carbon.

694

695 Table 5. Pearson correlations among CASH soil health indicators for mountain (Mills River) samples with
 696 $p < 0.05$ (bolded numbers $p < 0.01$; $n = 20$).

	AWC [†]	Surf Hard	Sub Hard	Ag Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
AWC															
SurfHard															
SubHard															
AgStab															
OM	0.65														
Protein	0.53			0.67	0.62										
Resp				0.46											
ActC				0.61		0.73	0.67								
pH							0.60	0.45							
P				0.46	0.47	0.70		0.78							
K				0.52	0.47	0.47	0.47	0.65		0.50					
Mg			-0.45		0.48	0.64	0.81	0.56	0.49	0.83					
Fe							-0.46	-0.50				-0.48			
Mn													-0.47		
Zn				0.66		0.68		0.60		0.73					

697 [†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard,
 698 penetration resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic
 699 matter; Resp, respiration during a 4-day incubation; ActC, active carbon.

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703 Table 6. P values for treatment effects on CASH soil health indicators for Goldsboro, Reidsville, and Mills River samples.

	AWC[†]	Surf Hard	Sub Hard	Agg Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
Goldsboro	NS [‡]	NS	NS	0.020	NS	NS	NS	0.072	NS	0.035	NS	NS	NS	NS	0.046
Reidsville	NS	NS	NS	0.006	NS	0.002	0.001	<0.001	0.01	0.06	0.008	0.016	NS	0.002	NS
Mills River	NS	NS	NS	0.052	NS	0.002	NS	<0.001	0.014	<0.001	0.002	0.011	0.075	NS	<0.001

[†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard, penetration resistance within the 15 to 45-cm zone; AgStab, water stable aggregation; OM, organic matter; Resp, respiration during a 4-day incubation; ActC, active carbon.

[‡]NS: not significant at $\alpha=0.1$.

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706 Table 7. Soil management contrasts related to CASH soil health indicators for Goldsboro, Reidsville, and Mills River samples. Bold
 707 numbers indicate treatment effects at $\alpha=0.05$. Of those, treatments within the same site followed by the same letter are not
 708 significantly different.

Site	AWC [†]	Surf Hard	Sub Hard	Agg Stab	OM	Protein	Resp	ActC	pH	P	K	Mg	Fe	Mn	Zn
Goldsboro															
ConvTill	0.160	1.72	2.877	13.07a	1.433	4.37	0.287	277	5.00	9.37ab	93.1	57.0	4.57	9.03	1.30
ConvTill-Org1	0.147	1.45	3.023	9.83ab	1.433	4.33	0.400	304	5.57	17.10a	122.3	77.3	5.97	5.80	2.93
ConvTill-Org2	0.167	1.15	2.563	6.60b	1.933	4.23	0.383	294	5.57	12.13ab	161.9	128.0	2.80	7.70	2.10
NoTill	0.183	1.78	2.520	14.40a	1.833	5.00	0.347	406	5.37	8.00b	108.9	111.0	6.10	7.13	1.30
p-value	0.707	0.584	0.768	0.020	0.589	0.110	0.426	0.072	0.183	0.035	0.118	0.396	0.479	0.720	0.046
Reidsville															
MoldboardPlow	0.149	2.00	3.75	6.45b	2.16	1.95c	0.229b	181b	5.39b	5.36	83.8b	103.3b	2.78	3.59b	1.663
ChiselTill	0.149	1.61	3.71	8.34b	2.44	2.95b	0.379a	313a	5.77a	9.96	116.4a	132.4ab	3.16	4.34b	1.450
MinimumTill	0.160	1.84	3.30	12.17a	2.77	4.43a	0.414a	372a	5.53ab	11.16	100.1ab	141.0a	3.00	5.14a	2.438
p-value	0.233	0.206	0.333	0.006	0.139	<0.001	0.001	<0.001	0.011	0.066	0.007	0.016	0.894	0.002	0.129
Mills River															
Conventional	0.207	1.655	2.760	11.73	2.532	3.79	0.375	277b	5.55	4.79b	90.9b	133.2b	5.34a	6.17	0.793
Organic	0.212	1.455	2.471	13.68	2.660	4.36	0.424	387a	5.78	9.22a	160.0a	179.1a	2.24b	7.09	0.950
p-value	0.428	0.474	0.080	0.572	0.582	0.233	0.317	0.003	0.139	0.011	<0.001	<0.001	0.039	0.252	0.210
ChiselTill	0.209	1.405	2.606	8.38b	2.481	3.40b	0.391	296	5.78	4.84b	124.4	153.1	3.98	6.29	0.588b
NoTill	0.211	1.705	2.625	17.03a	2.711	4.76a	0.408	367	5.56	9.18a	126.6	159.2	3.60	6.97	1.075a
p-value	0.694	0.277	0.914	0.003	0.314	<0.001	0.743	0.085	0.168	0.013	0.918	0.704	0.817	0.393	0.004

709 [†]AWC, available water capacity; SurfHard, penetration resistance within the 0 to 15-cm zone; SubHard, penetration resistance within the 15 to 45-cm zone;
 710 AgStab, water stable aggregation; OM, organic matter; Resp, respiration during a 4-day incubation; ActC, active carbon.

711

712 Table 8. Results for linear regression of mean corn and soybean yields on CASH soil health indicators, Reidsville site (all
713 $p < 0.05$; bolded: $R^2_{adj} > 0.75$). SD is the pooled standard deviation associated with annual yield variability.

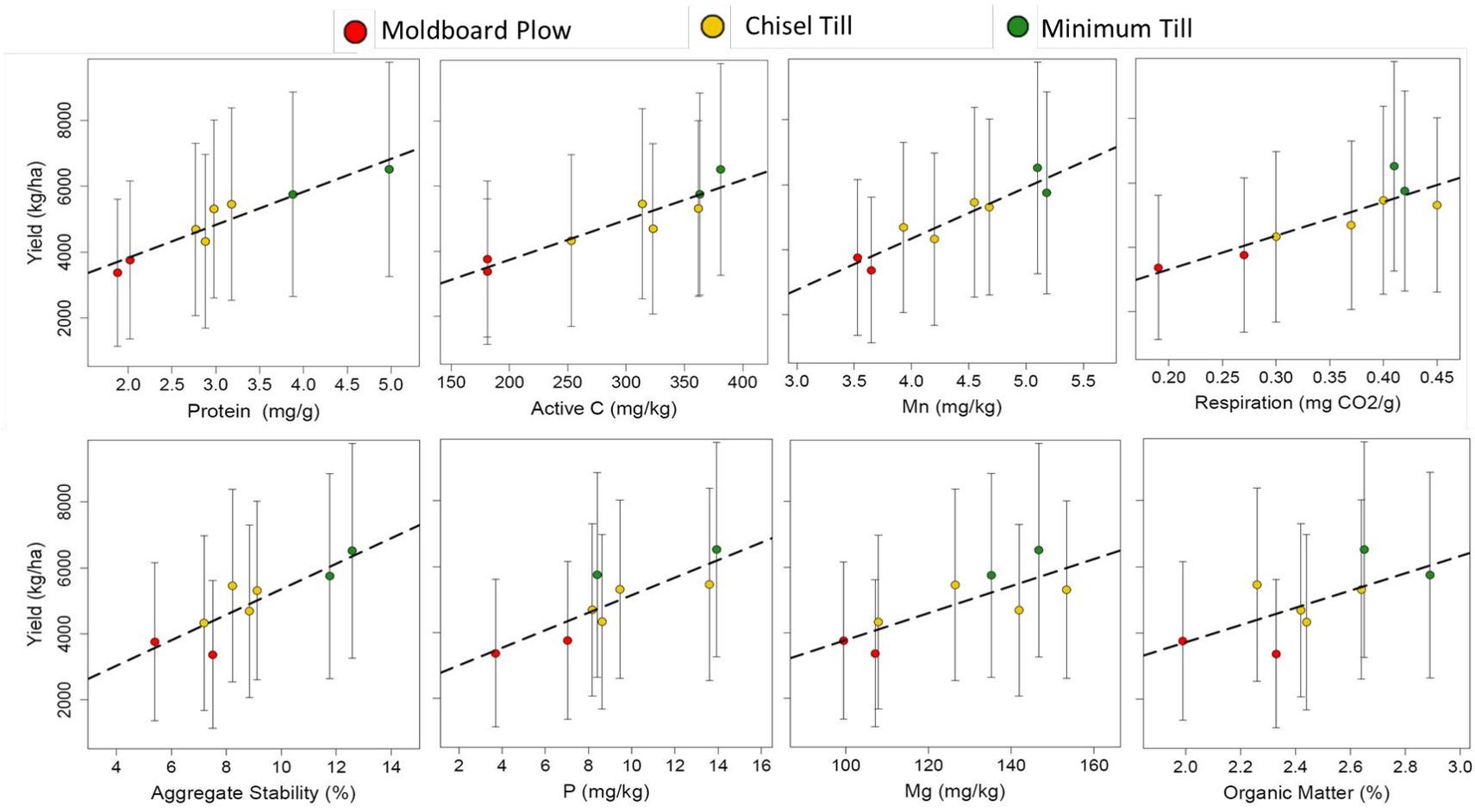
	AggStab[†]	OM	Protein	Resp	ActC	P	Mg	Mn
	%	%	mg g ⁻¹	mg CO ₂ g ⁻¹	----- mg kg ⁻¹ -----			
	<u>Corn Yield</u>							
R^2_{adj}	0.71	0.37	0.88	0.75	0.85	0.66	0.56	0.85
p-value, regr coeff	0.005	0.063	<0.001	0.003	<0.001	0.008	0.019	<0.001
intercept	1487	-1492	1822	1219	1284	2475	-333	-1946
slope	386	2606	1002	10478	12.26	266	41.1	1572
	<u>Soybean Yield</u>							
R^2_{adj}	0.48	0.31	0.55	0.90	0.93	0.59	0.76	0.65
p-value, regr coeff	0.033	0.090	0.021	<0.001	<0.001	0.015	0.003	0.01
intercept	1483	447	1616	1132	1207	1695	469	400
slope	113	831	283	3855	4.34	87	15.8	479

714 [†] AgStab, water stable aggregation; OM, organic matter; Resp, respiration during a 4-day incubation; ActC, active carbon.

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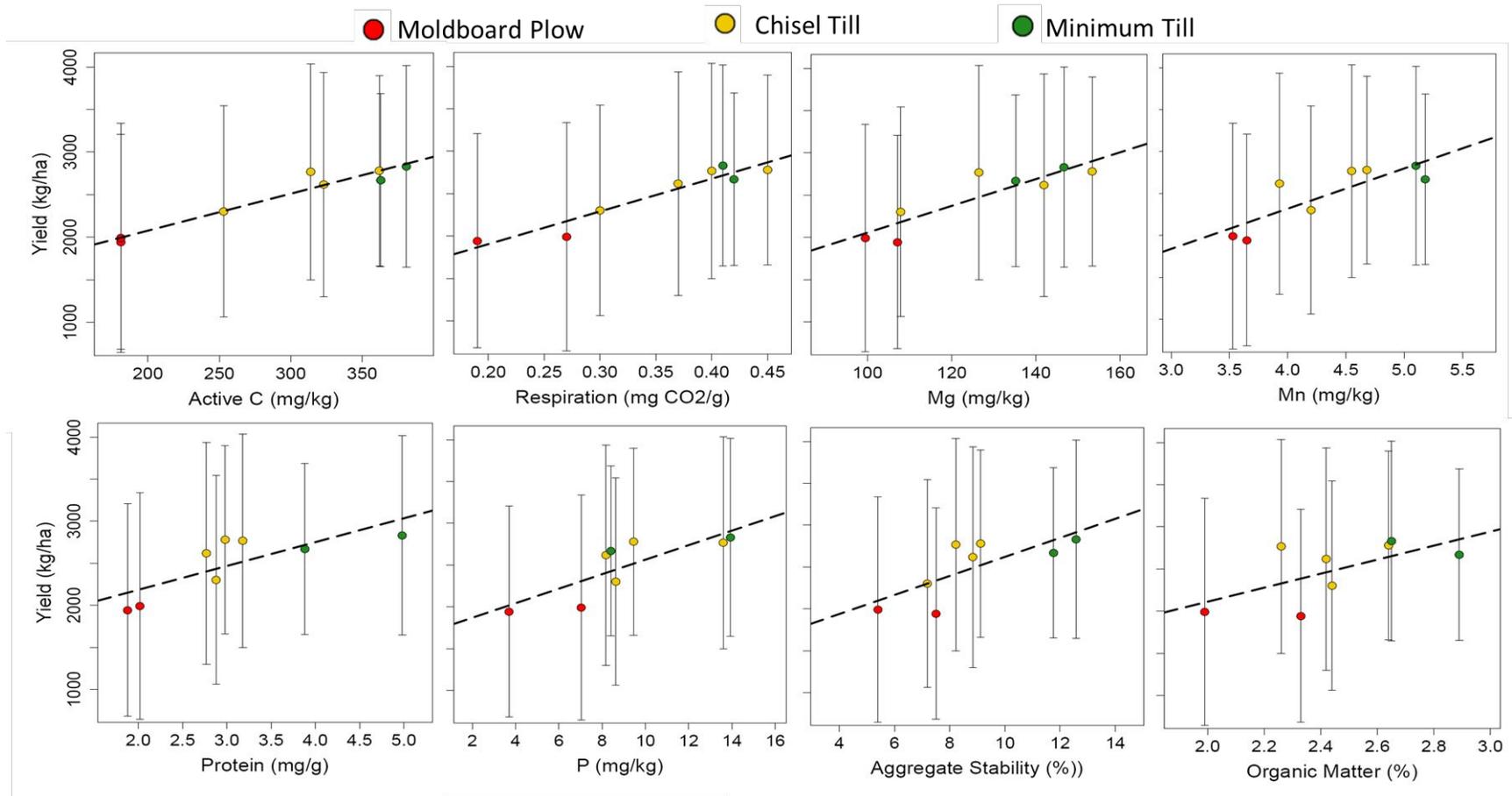


718

719 Figure 1. Linear regression of corn yield on CASH soil health indicators, organized from highest to lowest R^2_{adj} value (cf. Table 7). Regression line
 720 is based on mean yields for tillage treatments, and error bars represent standard deviations associated with annual yield variability.

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723

724 Figure 2. Linear regression of soybean yield on CASH soil health indicators, organized from highest to lowest R^2_{adj} value (cf. Table 7). Regression
 725 line is based on mean yields for tillage treatments, and error bars represent standard deviations associated with annual yield variability.

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