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Soil-Test Biological Activity with the Flush of CO_2: III. Corn Yield Responses to Applied Nitrogen

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Corn (*Zea mays* L.) is an important cereal grain in many states and typically receives large N fertilizer inputs, irrespective of historical management. Tailoring N inputs to soil-specific conditions would help to increase efficiency of N use and avoid environmental contamination. A total of 47 trials were conducted on research stations and private farms in four different regions of North Carolina and Virginia from 2014 to 2016 to associate soil N availability with yield response to sidedress N application. Corn grain yield was 10.6 ± 3.4 Mg ha^{-1} on 36 sites and silage yield was 44.6 ± 8.2 Mg ha^{-1} on 11 sites. There was positive association between relative yield (i.e., yield without sidedress N application divided by yield with full fertilization) and levels of both plant available N (residual inorganic N + net N mineralization during 24 d) and the flush of CO_2 following rewetting of dried soil during 3 d. Economically optimum N fertilizer requirement (EONR) at sidedress declined with increasing level of plant available N and soil-test biological activity (i.e., the flush of CO_2). The scalable N factor for production at EONR declined from 20 kg N Mg^{-1} of grain (i.e., 1.1 lb N bu^{-1}) with no soil biological activity to no N required with soil-test biological activity of 600 mg CO_2–C kg^{-1} in a 3-d period (depth of 0–10 cm). The flush of CO_2 when determined in spring at or prior to planting corn was considered an ideal soil-test indicator of soil biological activity due to its simple, rapid, and reliable characteristics related to potential soil N mineralization and corn yield responses to applied N fertilizer.

Abbreviations: EONR, economically optimum nitrogen fertilizer requirement; ISNT, Illinois soil nitrogen test; UAN, urea-ammonium nitrate.

Core Ideas

- **Relative corn yield was associated with residual inorganic and mineralizable N.**
- **Soil-test biological activity was a good surrogate for predicting N availability.**
- **Economically optimum N fertilizer requirement could be adjusted with soil testing.**

Annual corn (*Zea mays* L.) grain production is >250 Tg and silage production is >100 Tg in the United States (USDA-NASS, 2017a). Nearly all states in the United States have some corn production (except for Alaska; land area is as low as 100 ha in Rhode Island to as high as 5.5 million ha in Iowa; total of 35 million ha for grain in the United States). Corn is an important food, feed, and biofuel crop.

Nitrogen application to corn is based on historical dogma that suggests N removed from the field in grain must be replaced (i.e., ~1% N concentration of grain; 10 kg N Mg grain^{-1}). Nitrogen contained in residual stover is also considered in need of replacement (i.e., ~1% N concentration of stover that is ~50% of total biomass; 10 kg N Mg stover^{-1}), as it is not readily available or subsequently lost through gas and/or leaching. This would effectively result in 20 kg N Mg grain^{-1}. University recommendations vary slightly, but targets of 17.9 to 21.4 kg N Mg grain^{-1} (1.0–1.2 lb N bu^{-1}) encompass most recommendation systems in the eastern United States (Beegle and Durst, 2003; Alley et al., 2009; Mitchell and Huluka, 2012; Harris, 2017). Silage production systems often have similar recommendations with different units (17.9–21.4 kg N Mg silage^{-1} or 8–10 lb N t silage^{-1}). Empirical data from field trials in North Carolina have been assembled over a number of years on different soil types to form the scientific basis for recommending N (Rajkovich et al., 2015). Both

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theoretical and empirical approaches result in similar N recommendations, at least in North Carolina.

The N factor approach (i.e., determining the quantity of N recommended per unit of production) allows for some flexibility to make adjustments for variations in management differences. For example, soybean as previous crop to corn is given credit of 25 kg N ha^{-1} in North Carolina (Rajkovich et al., 2015), although the N credit is not proportional to actual yield level of corn. Use of no-till rather than conventional tillage also can lead to adjustment of the N factor. All of these adjustments require sufficient empirical data for support. A myriad of different management considerations can be expected on individual farms within a state, especially in the eastern United States where farms have a diversity of crop rotations. An alternative and more theoretical basis for N fertilizer adjustments based on soil testing would allow for greater precision in defining N requirements on individual farms. Such an approach assumes that management differences would actually have a discernable effect on some measurable component of the soil.

A long history has evolved for trying to develop a soil testing approach for N management (Hatfield and Follett, 2008). Early research in soil fertility prior to industrial fertilizer sources focused on soil biological activity (Waksman, 1916; Lebedjantzev, 1924). Good results existed for defining soil fertility by the amount of soil microbial activity through respiration (Waksman and Starkey, 1924). With the development of the Haber-Bosch process for industrial fixation of N, much of the research on natural soil fertility assessments was abandoned. Large volumes of research papers since the 1960s have focused on defining the amount of commercial N needed to maximize yields (Russell, 1963; Triplett et al., 1979; Jokela and Randall, 1989; Stecker et al., 1995; Shapiro and Wortman, 2006). More recent research has rightly shifted the focus toward economically optimum N requirement (EONR), a concept that recognizes the cost of N fertilizer inputs has to be met with at least an equivalent value of harvestable and valued product (Vanotti and Bundy, 1994; Scharf et al., 2005; Williams et al., 2007). Widely shifting economics of input costs and commodity prices during the past decade suggest that even greater emphasis will be needed to balance short-term economic indicators with long-term goals of sustainability (Wright, 2011; Etienne et al., 2016).

Defining soil N mineralization has been a central focus of an N-balance approach to economical crop production (Griffiths and Robinson, 1992; Brisson et al., 1998; Ross et al., 2008). Stanford and Smith (1972) developed an approach to characterize potentially mineralizable N based on several months of incubation. Stanford et al. (1973) used greenhouse growth trials to associate plant N uptake with net N mineralization in the laboratory with very good results. However, relationship of net N mineralization to plant N uptake in the field has not always been so clear. Several reasons are possible for discrepancies between laboratory-determined N mineralization and field-determined yield responses to N. One important consideration is the variable weather that ensues in the field following soil sampling. Soil temperature and water content are key controlling factors of N mineralization and subsequent potential N losses to the environment via volatile, soluble, and particulate flow mechanisms that limit plant N uptake. Another reason may be immobilization of mineralized N (Wang et al., 2001), either by soil microorganisms, their reactive substrates, or inorganic colloids. Also, there can be discrepancy of determining net N mineralization from surface soil samples only, while plants grown in the field have access to deeper soil depths and variable and often undetermined supply of residual inorganic N.

Recent research has focused more on rapid indicators of soil N availability to crops (Khan et al., 2001). From ~100 farm fields in Illinois, determination of amino-sugar N concentration (i.e., the Illinois Soil Nitrogen Test [ISNT]) was able to differentiate between N-responsive and non-responsive sites (Mulvaney et al., 2006). From 33 field trials in New York, pre-sidedress nitrate test (or PSNT), and ISNT when combined with organic matter concentration, were able to distinguish between N-responsive and non-responsive sites (Klapwyk and Ketterings, 2006). The ISNT was also inversely related with EONR within well-drained and poorly drained soil classes (separately, but not together) in North Carolina (Williams et al., 2007). In a subsequent study of variations in ISNT with time, depth, and management in soils of North Carolina, EONR varied up to 80 kg N ha^{-1} with small variations in ISNT (Wall et al., 2010), suggesting that variations in ISNT may not be directly proportional with N mineralization. From 43 trials in Iowa, ISNT concentration was not able to differentiate between sites that were N-responsive and non-responsive and it had poor association with EONR (Barker et al., 2006).

Most soil N availability indicators are chemical extractions that target the labile component of soil organic matter (Schomberg et al., 2009). An alternative approach has returned to soil biological activity as an indicator of soil fertility (Franzluebbers et al., 1996, 2000). Good relationships have now been established between the flush of CO_2 following rewetting of dried soil and net N mineralization (Franzluebbers and Brock, 2007; Franzluebbers and Stuedemann, 2008; Franzluebbers and Haney, 2017). Use of dried soil and the rapid nature of the assessment process has led to commercial development of a soil test, albeit using different detection and water delivery methodologies, and shorter incubation time (Haney et al., 2008; Haney and Haney, 2010).

Given recent evidence of strong correlation of the flush of CO_2 with net N mineralization (Franzluebbers et al., 2018) and effective relationship with total N uptake by plants growing in the greenhouse (Franzluebbers and Pershing, 2018), this study was designed to test if soil-test biological activity could relate to N availability in the field by determining corn yield responses to N fertilizer inputs in a variety of soils and farms having different management histories. The hypothesis was that soil-test biological activity would be low in those fields having high yield response to N fertilizer inputs and would be high in those fields having low yield response to N fertilizer inputs. A large number of observations across a gradient of soil-test biological activity levels was needed to be able to make such an association, given historical indications

of large yield variations among fields with different histories (i.e., the reality of a recommendation domain). Soil sampling depth was also tested for its influence in defining such an association.

MATERIALS AND METHODS

Soil samples were collected from a total of 47 fields in North Carolina and Virginia (Table 1). Sites were sampled that were in corn production in 2014, 2015, or 2016. Sites were located in four distinct physiographic regions, including the Coastal Plain (9 sites), Piedmont (15 sites), Great Valley (20 sites), and Blue Ridge (3 sites). Long-term mean annual temperature ranged from 11.2 to 16.0°C and precipitation ranged from 859 to 1300 mm.

Soil was typically collected from four replicate blocks within both research station trials (4 sites and total of 11 site-years) and private farms (19 sites and total of 36 site-years). Soils were sampled at depths of 0 to 10, 10 to 20, and 20 to 30 cm with a hydraulic probe (4-cm i.d.) or same-sized hand probe. Typically, eight cores within a block were composited by depth in a paper bag, transported to the laboratory, and dried by placing in an oven at 55°C for ≥3d until constant weight (sometimes initially by blowing room-temperature air over the sample on a paper plate followed by oven drying). Soil was then gently crushed with pestle over a screen with 4.75-mm openings. Stones and residues not passing the screen were weighed (if greater than ~5% of total weight) and removed from the sample during further processing. Methods of soil analysis and their results were presented in two previous reports in this series (Franzluebbers and Pershing, 2018; Franzluebbers et al., 2018), and therefore only brief descriptions are reported here.

Total organic C and N were determined with dry combustion using a Leco TruMac CN analyzer. Routine soil nutrient analyses (soil pH, acidity, cation exchange capacity, base saturation, and extractable Ca, Cu, K, Mg, Mn, Na, P, S, Zn) were conducted by Soil Testing Services of the North Carolina Department of Agriculture and Consumer Services in Raleigh, NC. Soil organic C and N fractions were determined according to Franzluebbers and Stuedemann (2008). Soil microbial biomass C was determined with chloroform fumigation-incubation without subtraction of a control and using an efficiency factor of 0.41 (Voroney and Paul 1984; Franzluebbers et al., 1999). The flush of CO_2 following rewetting of dried soil (3 d) and cumulative C and N mineralization during 24 d of incubation were determined with aerobic incubation of soil at 50% water-filled pore space and 25°C (Franzluebbers, 2016). Inorganic N was determined by auto-analyzer techniques of filtered extracts from 10 g dried soil and 20 mL of 2 mol L^{-1} KCl. Plant-available N was calculated as the summation of residual inorganic N (NO_3 + NH_4) and mineralizable N during 24 d of incubation. Particulate organic C and N were determined from dry combustion of the sand-sized fraction. From greenhouse growth trials, plant response indicators (i.e., dry matter and N uptake from unamended soils at each depth) were used as an integrative characteristic (Franzluebbers and Pershing, 2018).

Concentrations of soil properties were also calculated across cumulative depths of 0 to 20 and 0 to 30 cm. This required adjustment by soil density in each layer. Soil bulk density was assumed from a pedotransfer function related to soil organic C concentration (Franzluebbers and Stuedemann, 2010):

$$BD = 1.75 \exp(-0.0132\ SOC)$$

where BD is bulk density (Mg m^{-3}) and SOC is soil organic C (g kg^{-1}).

Yield response trials were conducted on fields in collaboration with local field personnel. Each individual trial was designed to test whether additional N applied at sidedress (~V6; except two trials applied at planting) would yield greater grain or silage dry matter, and if so, how much more yield per unit of applied N. Three general types of field designs were conducted. Replicated, small-plot trials were conducted at 15 sites (Table 1). Six trials in Henderson, Lenoir, and Rowan Counties of North Carolina were conducted by project collaborators with a randomized, complete block design using multiple N rates (0, 45, 90, 134, 179, and 224 kg N ha^{-1}) applied as urea-ammonium nitrate (UAN) in plots of 3 m by 10 m each and replicated four times. Six trials in Camden, Henderson, and Washington Counties of North Carolina were conducted by a project collaborator using a randomized, complete block design with three N rates (0, 84, and 168 kg N ha^{-1}) applied as UAN and replicated four times. Plots chosen had no N applied at planting and yield with sidedress N application at V6 and V12 were averaged. Three replicated, small-plot trials in Augusta County VA were implemented by project collaborators with a randomized, complete block design using three N rates (0, 50, and 100 kg N ha^{-1}) applied as urea at sidedress in plots of 4.5 m by 9 m and replicated four times. Replicated strip trials were conducted at 8 sites (Table 1). These trials were implemented by farmers and/or research-station staff and consisted of ≥9-m-wide by ≥50-m-long strips with three N rates (typically 0, 50, and 100 kg N ha^{-1}) applied as typically UAN and replicated 2 to 3 times (two sites at planting and six sites at sidedress). Unreplicated, field-length strips were conducted at 24 sites (Table 1). These trials were implemented by farmer collaborators and consisted typically of ≥9-m-wide by ≥200-m-long strips with at least three N rates (low rate was typically no N, medium rate was 40 to 100 kg N ha^{-1}, and high rate was 100 to 200 kg N ha^{-1}) applied as UAN (all sites at sidedress).

Corn grain (36 sites) and silage (11 sites) yields were obtained with a variety of approaches that matched field design and interests of investigators and/or collaborators. Replicated, small-plot trials were typically harvested by hand from one to two of the center rows of 3- to 5-m length, ears dried, and grain shelled, weighed, and adjusted to 15.5% moisture. Replicated strip trials were mostly harvested by hand as well from one row of 3.8-m length at six separate locations spaced equally along the length of a strip (sequencing diagonally across the middle 6 rows). Unreplicated, field-length strips were harvested by hand from one row of 3.8-m length at 12 separate locations spaced equally along the length of a strip (sequencing diagonally across the middle 8 rows of a 12-row strip).

Table 1. Location, management, soil texture, and precipitation (May to September) at field sites across several counties in North Carolina and Virginia.

Code	Location	Tillage†	Previous crop	Soil series and textural class‡	Precip.§	Design¶
					mm	
			Coastal Plain			
LCPS4	Lenoir Co NC	DT	Soybean	Lynchburg SL	982	RSP
LCPS5	Lenoir Co NC	DT	Soybean	Portsmouth L	637	RSP
NCCC5	Camden Co NC	DT	Corn	Perquimans SiL	360	RSP
NWZC6	Halifax Co NC	NT+IRS (Irr)	Corn + multi-species cover	Nahunta SiL	NA	UFLS
NWZS6	Halifax Co NC	NT+IRS (Irr)	Soybean + multi-species cover	Grantham L	NA	UFLS
TRS15	Washington Co NC	DT (Irr)	Soybean	Cape Fear L	554	RSP
TRS25	Washington Co NC	NT	Soybean	Portsmouth fSL	554	RSP
TRSD4	Washington Co NC	DT	Soybean	Portsmouth fSL	420	RSP
TRSI4	Washington Co NC	DT (Irr)	Soybean	Portsmouth fSL	420	RSP
			Piedmont			
NLNL5	Stanly Co NC	NT	Corn	Chewacla L	379	UFLS
NLNN6	Montgomery Co NC	NT	Soybean	Tillery SiL	609	UFLS
NLNP6	Montgomery Co NC	PT	Soybean	Creedmoor-Brickhaven complex	609	UFLS
NLNU5	Stanly Co NC	NT	Cotton + multi-species cover	Tarrus channery SiCL, Georgeville SiCL	379	UFLS
NPJ_6	Stanly Co NC	NT	Cotton + rye cover	Tarrus channery SiL, Badin channery SiL	609	UFLS
PRSB5	Rowan Co NC	NT	Corn + heavy poultry litter	Mecklenburg CL	214	RS
PRSB6	Rowan Co NC	NT	Corn + rye cover + poultry litter	Mecklenburg CL	486	RS
PRSD5	Rowan Co NC	NT	Corn + barley cover + dairy slurry	Lloyd CL, Dorian fSL	214	RS
PRSD6	Rowan Co NC	NT	Corn + multi-species cover	Lloyd CL, Dorian fSL	486	RS
PRSS4	Rowan Co NC	NT	Soybean	Lloyd CL	NA	RSP
PRSS5	Rowan Co NC	NT	Soybean	Lloyd CL	214	RSP
VBFS6	Culpeper Co VA	NT	Soybean	Rapidan-Penn complex	424	UFLS
VBH35	Fauquier Co VA	NT	Soybean + sparse multi-species cover	Ashburn SiL	337	UFLS
VBH65	Fauquier Co VA	NT	Corn + rye cover	Ashburn SiL, Dulles SiL, Albano SiL	337	UFLS
VSK_5	Fauquier Co VA	NT	Corn silage + dairy manure	Ashburn SiL, Dulles SiL, Albano SiL	337	UFLS
			Great Valley			
VBAA5	Rockingham Co VA	NT	Corn and barley silage + dairy manure	Frederick SiL, Lodi SiL	388	RS
VBJ_5	Augusta Co VA	NT	Soybean + rye cover + poultry litter and beef bedpack	Edom SiL	388	RS
VBJ_6	Augusta Co VA	NT	Soybean	Edom SiL	517	RS
VCKA5	Augusta Co VA	NT	Corn + multi-species cover	Frederick SiL, Lodi SiL	388	RS
VDMN6	Augusta Co VA	NT	Soybean + poultry litter	Endcav SiL	517	UFLS
VDMS6	Augusta Co VA	NT	Soybean + poultry litter and biological amendments	Endcav SiL	517	UFLS
VGE_5	Augusta Co VA	NT	Corn + dairy manure	Frederick-Christian SiL	388	UFLS
VGE_6	Augusta Co VA	NT	Corn	Frederick-Christian SiL	517	UFLS
VHNA5	Augusta Co VA	NT	Corn + multi-species cover	Sequoia-Berks SiL	388	UFLS
VKJ_5	Rockingham Co VA	NT	Corn + multi-species cover	Craigsville cobbly fSL	388	UFLS
VLK_5	Augusta Co VA	NT	Corn and barley silage + dairy slurry	Frederick-Christian SiL	388	UFLS
VRD_5	Augusta Co VA	NT	Corn + rye cover + poultry litter	Chagrin L	388	UFLS
VRD_6	Augusta Co VA	TT	Corn	Chagrin L	517	UFLS
VRM_5	Augusta Co VA	NT	Corn silage + multi-species cover	Chavies fSL	388	UFLS
VRM_6	Augusta Co VA	NT	Corn silage + multi-species cover	Chavies fSL	517	RSP
VSD_5	Augusta Co VA	NT	Corn silage + multi-species cover	Frederick-Christian SiL	388	UFLS
VSD_6	Augusta Co VA	NT	Soybean + poultry litter	Frederick-Christian SiL	517	UFLS
VSR_5	Rockingham Co VA	NT	Barley silage + sorghum-sudangrass hay	Frederick SiL, Lodi SiL	388	UFLS
VSRL6	Rockingham Co VA	NT	Corn + multi-species cover	Craigsville cobbly fSL	517	RSP
VSRU6	Rockingham Co VA	NT	Corn silage + multi-species cover	Frederick SiL, Lodi SiL	517	RSP
			Blue Ridge			
MHRS4	Henderson Co NC	DT	Wheat	Comus fSL	611	RSP
MHRS5	Henderson Co NC	DT	Corn	Codorus L	546	RSP
MHRC5	Henderson Co NC	DT	Corn	Comus fSL	546	RSP

† DT, disk tillage; IRS, in-row subsoil; NT, no tillage; PT, plow tillage; TT, turbo tillage (shallow vertical tillage); Irr, irrigated.

‡ CL, clay loam; fSL is fine sandy loam; L, loam; SiL, silt loam; SL, sandy loam.

§ Precipitation was from nearest weather station possible (NA indicates not available).

¶ Trial designs: RS, replicated strips; RSP, replicated small plots; UFLS, unreplicated field-length strips.

Corn silage yield was determined at one site by weighing the entire strip contents using commercial scale and adjusting to 35% moisture. At 10 sites, silage yield was determined by hand harvest of one row of 3.8-m length cut at approximately 15-cm height at 12 separate locations within a strip, weighing the contents in the field on a hanging scale, randomly selecting three plants to be weighed in the field and after drying at 60°C, and then calculating dry matter adjusted to 35% moisture. Variation in types and number of replicate plots was not considered a hindrance, because regression of yield on N rate was across replicate data for each site.

Yield estimates for statistical evaluation were obtained from (a) individual plots of N-rate treatments in replicated small-plot trials and (b) average of three neighboring locations within a field-length strip in replicated strip trials and in unreplicated field-length strips to create four observations of each N-rate treatment. Regression of yield on multiple N rates was determined across the four replications for each of the 47 field trials. All data were first tested for fit to a nonlinear function of the form:

$$Y = Y_0 + a\,(1 - \exp[-bN])$$

where Y is yield (Mg ha^{-1}), Y_0 is the baseline yield without N (Mg ha^{-1}), a is the additional yield potential with limitless N input (Mg ha^{-1}), b is a nonlinear rate constant, and N is the N rate (kg N ha^{-1}). When the nonlinear equation produced a negative yield response or an unrealistically rapid rise at the first instance of N input followed by no change thereafter, then a linear regression was applied to the data. If the linear regression had negative slope, then mean yield of the N rates was calculated to assume no response to N input. These were the only choices used to calculate the following parameters of interest for further statistical evaluation of each site: (a) maximum yield based on regression at the highest N rate tested (Mg ha^{-1}); (b) relative yield without N fertilizer derived from the best-fit regression equation at 0 kg N ha^{-1} divided by maximum yield (Mg Mg^{-1}); (c) yield response to initial dose of N (empirically derived from the instantaneous yield produced at the first instance of N, i.e., the product of regression parameters a and b based on best-fit regression (kg yield kg N^{-1}); (d) economically optimum N fertilizer requirement (EONR) at low cost-to-value ratio (kg N ha^{-1}); (e) EONR at medium cost-to-value ratio (kg N ha^{-1}); and (f) EONR at high cost-to-value ratio (kg N ha^{-1}). Threshold cost-to-value ratios were calculated from the cost of N fertilizer (US$ kg^{-1}) and value of corn yield ($ kg^{-1}). Low threshold cost-to-value ratio was calculated at equivalent of $1.00 kg N^{-1} and $0.20 kg grain^{-1} (5 kg grain kg N^{-1}) or $1.00 kg N^{-1} and $0.04 kg silage^{-1} (25 kg silage kg N^{-1}). High threshold cost-to-value ratio was calculated at equivalent of $2.00 kg N^{-1} and $0.10 kg grain^{-1} (20 kg grain kg N^{-1}) or $2.00 kg N^{-1} and $0.02 kg silage^{-1} (100 kg silage kg N^{-1}). Medium threshold cost-to-value ratio was calculated similarly for targets of 10 kg grain kg N^{-1} and 50 kg silage kg N^{-1}. These three targets of EONR (kg N ha^{-1}) were also calculated as N factor for economically optimum production (kg N Mg grain^{-1} or kg N Mg silage^{-1}).

Plant response variables were single observations for each site. Soil variables were also averaged across replications to associate with plant variables. Standard deviation of soil variables were calculated for each of the 47 trials when needed. Linear and nonlinear regressions were performed with means from each site using SAS v. 9.4 and SigmaPlot v. 13. To show more clearly the trends in corn grain yield response data amid the large field variations, means of six consecutive sites in ranked order of soil-test biological activity were calculated and used in linear regressions (n = 6 sites in each group). Regressions for relative yield were combined across grain and silage trials, while all other yield response components had to be separated for grain and silage trials due to incongruous yield units. Data were plotted with SigmaPlot v. 13. Effects were considered significant at $p \le 0.05$. Significance of correlations among variables was set at a stricter threshold of $p \le 0.01$ to avoid spurious associations.

RESULTS AND DISCUSSION

Corn was reasonably productive at most locations for both grain (Table 2) and silage (Table 3). Maximum grain yield was greatest in the Blue Ridge (15.7 ± 2.6 Mg ha^{-1}) and lowest in the Piedmont (9.0 ± 1.8 Mg ha^{-1}). Grain yields in the Coastal Plain and Great Valley were intermediate. Adverse weather conditions contributed to low grain production at a few sites, including three dryland sites in Washington County NC (5.0 ± 1.9 Mg ha^{-1}) and two dryland sites in Rowan County NC in 2015 (6.3 ± 0.4 Mg ha^{-1}). Median grain yield across all 36 sites was 10.2 Mg ha^{-1} and data were normally distributed. Maximum silage yield was similar in the Piedmont (47.7 ± 8.8 Mg ha^{-1}) and Great Valley (43.9 ± 8.5 Mg ha^{-1}). State-wide corn grain yields were 8.0 and 9.3 Mg ha^{-1} in North Carolina and Virginia in 2016 and silage yields were 34.7 and 44.8 Mg ha^{-1} in North Carolina and Virginia in 2016 (USDA-NASS, 2017b, 2017c), and therefore these trials provided a reasonable representation of typical production in the region.

The majority of field tests showed significant N limitation to achieve optimum production for grain (Table 2), but not for silage (Table 3). Of the 36 sites evaluated for grain production, 23 of the sites produced <90% of maximum yield without sidedress N application. Of the 11 sites evaluated for silage production, only 3 of the sites produced <90% of maximum yield without sidedress N application. Therefore, a diversity of sites was

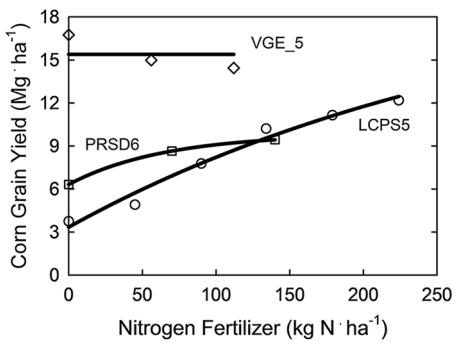

Fig. 1. Examples of corn grain yield response to N at three sites.

Table 2. Grain yield response and N characteristics of each site sorted by physiographic region.

Field code	Model type†	Regression parameter‡				Maximum yield	No. of rates	Relative yield	EONR§	Yield response at initial dose of N	N factor
		Y_0	a	b	r^2						
		Mg ha^{-1}				Mg ha^{-1}		Mg Mg^{-1}	kg N ha^{-1}	kg grain kg N^{-1}	kg N Mg grain^{-1}
Coastal Plain											
LCPS4	NL	4.9	11.5	0.0072	0.97	14.1	6	0.35	224	83	15.9
LCPS5	NL	3.8	17.8	0.0036	0.97	13.6	6	0.28	224	64	16.5
NCCC5	NL	11.4	4.8	0.0222	0.65	16.1	3	0.71	139	107	8.6
NWZC6	NL	6.8	6.3	0.0121	0.88	12.9	4	0.53	226	76	17.6
NWZS6	NL	5.8	7.8	0.0098	0.89	13.0	4	0.44	276	76	21.2
TRS15	L	2.3	1	0.0098	0.49	4.0	3	0.58	168	10	42.4
TRS25	M	3.8	0	0	0	3.8	3	1.00	0	0	0
TRSD4	L	6.8	1	0.0024	0.02	7.2	3	0.95	0	2	0
TRSI4	NL	5.7	6.9	0.0180	0.82	12.3	3	0.47	168	124	13.7
Mean		5.7	6.3	0.0095	0.64	10.8	4	0.59	158	60	15.1
Piedmont											
NLNL5	NL	10.7	1.8	0.0456	0.22	12.4	3	0.86	60	80	4.9
NLNN6	L	7.9	1	0.0042	0.23	8.5	3	0.93	0	4	0
NLNP6	NL	9.2	1.4	0.0252	0.16	10.5	3	0.88	76	34	7.3
NLNU5	NL	7.8	2.1	0.0227	0.25	9.7	3	0.81	99	47	10.2
NPJ_6	L	4.0	1	0.0241	0.77	9.4	3	0.43	224	24	23.9
PRSB5	M	7.2	0	0	0	7.2	2	1.00	0	0	0
PRSB6	M	6.6	0	0	0	6.6	3	1.00	0	0	0
PRSD5	M	9.9	0	0	0	9.9	2	1.00	0	0	0
PRSD6	NL	5.4	3.0	0.0155	0.32	8.0	3	0.67	140	46	17.5
PRSS4	NL	5.9	5.7	0.0132	0.80	11.3	6	0.52	205	75	18.1
PRSS5	NL	2.7	4.2	0.0070	0.60	6.0	6	0.45	224	30	37.1
VBFS6	NL	5.7	3.0	0.0193	0.46	8.6	3	0.66	127	57	14.7
VBH35	NL	5.1	6.2	0.0062	0.17	8.4	3	0.60	125	38	14.9
Mean		6.8	2.2	0.0141	0.33	9.0	3	0.75	98	33	11.4
Great Valley											
VBJ_5	M	16.6	0	0	0	16.6	3	1.00	0	0	0
VBJ_6	L	8.8	1	0.0010	0.00	8.9	3	0.99	0	1	0
VCKA5	NL	10.7	0.7	0.0139	0.56	11.2	3	0.95	43	9	3.8
VDMN6	L	7.6	1	0.0218	0.75	11.1	3	0.69	157	22	14.2
VDMS6	NL	9.0	3.2	0.0066	0.79	11.1	3	0.82	157	21	14.2
VGE_5	M	9.0	0	0	0	9.4	3	1.00	0	0	0
VGE_6	L	8.3	1	0.0020	0.06	8.3	3	1.00	0	2	0
VHNA5	NL	11.2	2.2	0.0120	0.39	12.8	3	0.87	112	26	8.7
VRD_5	L	12.5	1	0.0121	0.14	14.1	3	0.89	134	12	9.5
VRD_6	M	7.7	0	0	0	7.7	3	1.00	0	0	0
VSD_6	L	9.3	1	0.0024	0.02	9.7	3	0.96	0	2	0
Mean		10.1	1.0	0.0065	0.28	11.0	3	0.92	55	9	4.6
Blue Ridge											
MHRC5	NL	6.3	12.5	0.0043	0.70	12.7	3	0.49	168	54	13.2
MHRS4	NL	9.5	7.0	0.0258	0.86	16.5	6	0.58	139	181	8.4
MHRS5	NL	3.6	35.0	0.0023	0.93	17.8	6	0.20	224	82	12.6
Mean		6.5	18.2	0.0108	0.83	15.7	5	0.42	177	105	11.4

† First choice of model was nonlinear (NL), second choice was linear (L), and third choice was mean of observations (M).

‡ Regression parameters describe the nonlinear form as $Y = Y_0 + a\,(1 - \exp[-bN])$ and the linear and mean forms as $Y = Y_0 + abN$ where N is the N rate (kg N ha^{-1}).

§ EONR, economically optimum N fertilizer rate; EONR is derived from the regression equation and low cost-to-value threshold of 5 kg grain kg N^{-1}.

obtained (and was needed to meet the goal) to assess soil limitations on N availability. Figure 1 shows examples of the types of yield responses to N fertilizer application that occurred among sites. As a summary, relative yield was 0.42 ± 0.20 for grain in the Blue Ridge, 0.59 ± 0.25 for grain in the Coastal Plain, 0.75 ± 0.21 for grain in the Piedmont, 0.92 ± 0.10 for grain in the Great Valley, 0.96 ± 0.01 for silage in the Piedmont, and 0.95 ± 0.06 for silage in the Great Valley. Although there were regional differences, there was also wide enough variation within a region to allow for overlapping responses across regions.

Relative yield of both grain and silage without sidedress N application was related with plant available N (i.e., residual inorganic N + mineralizable N during 24-d aerobic incubation) at a depth of 0 to 30 cm (Fig. 2). Strength of the relationship was only moderate when all available data were included (r^2 = 0.31, n = 45). When 5 sites were excluded that had low yield

Table 3. Silage yield response and N characteristics of each site sorted by physiographic region.

Field code	Model type†	Regression parameter‡				Maximum yield	No. of rates	Relative yield	EONR§	Yield response at initial dose of N	N factor
		Y_0	a	b	r^2						
		Mg ha^{-1}				Mg ha^{-1}		Mg Mg^{-1}	kg N ha^{-1}	kg silage kg N^{-1}	kg N Mg silage^{-1}
Piedmont											
VBH65	L	51.7	1	0.0173	0.03	53.9	3	0.96	0	17	0
VSK_5	L	39.7	1	0.0159	0.01	41.4	3	0.96	0	16	0
Mean		45.7	1.0	0.0166	0.02	47.7	3	0.96	0	17	0
Great Valley											
VBAA5	M	48.6	0	0	0	48.6	3	1.00	0	0	0
VKJ_5	M	43.6	0	0	0	43.6	3	1.00	0	0	0
VLK_5	L	48.5	1	0.0419	0.20	56.0	3	0.87	179	42	3.2
VRM_5	NL	37.0	1	0.0180	0.13	40.3	3	0.92	0	18	0
VRM_6	L	36.4	1	0.0408	0.11	41.0	3	0.89	112	41	2.7
VSD_5	M	56.9	0	0	0	56.9	3	1.00	0	0	0
VSR_5	L	27.6	1	0.0501	0.35	30.9	2	0.89	67	50	2.2
VSRL6	L	39.1	1	0.0094	0.01	40.2	3	0.97	0	9	0
VSRU6	M	37.9	0	0	0	37.9	3	1.00	0	0	0
Mean		41.7	1.0	0.0178	0.02	43.9	3	0.95	40	18	0.9

† First choice of model was nonlinear (NL), second choice was linear (L), and third choice was mean of observations (M).

‡ Regression parameters describe the nonlinear form as $Y = Y_0 + a\,(1 - \exp[-bN])$ and the linear and mean forms as $Y = Y_0 + abN$ where N is the N rate (kg N ha^{-1}).

§ EONR, economically optimum N fertilizer rate; EONR is derived from the regression equation and low cost-to-value threshold of 25 kg silage kg N^{-1}.

due to weather and/or unrealistic linear yield response, strength was nearly doubled ($r^2 = 0.60$, $n = 40$). The association between relative yield and plant available N was statistically significant and followed an expected nonlinear pattern of large changes in relative yield with lowest plant available N level and small changes in relative yield with highest plant-available N levels. When soil sampling was considered for a depth of 0 to 20 cm, then the coefficient of determination increased slightly to 0.64. At even shallower sampling depth of 0–10 cm, relative yield (RY) was equally well related with plant available N (PAN), according to the following:

$$RY = -0.22 + 1.38\,(1 - \exp[-0.01053\,PAN]),$$
$$r^2 = 0.64\,(p < 0.001),\ n = 42$$

The proportion of plant available N as residual inorganic N at depth of 0 to 30 cm was 0.20 ± 0.08 kg kg^{-1} across sites.

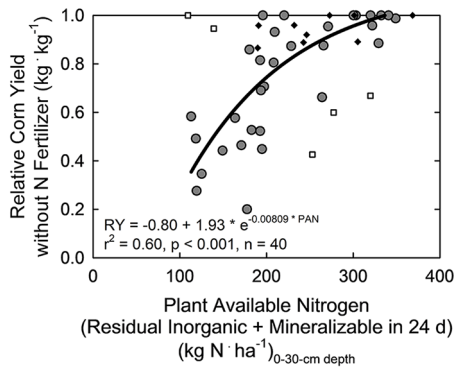

Fig. 2. Relative yield of corn (grain [filled circles] and silage [filled diamonds]) without sidedress N application to that of maximum yield with sufficient N as related to plant available N in the soil. Two sites did not have soil analyses at 20- to 30-cm depth and five sites (open squares) were removed from the regression due to concerns about low yield and linear yield response.

The proportion did not change substantially when soil sampling depth was restricted, but residual inorganic N varied with depth from 26 ± 16 kg N ha^{-1} at 0 to 10 cm, 37 ± 19 kg N ha^{-1} at 0 to 20 cm, and 47 ± 22 kg N ha^{-1} at 0 to 30 cm. The possibility cannot be excluded that significantly greater residual inorganic N may have been present deeper in the soil profile (i.e., below 30-cm depth), as soil measurements below this depth were not made. Soil-profile nitrate N can contribute significantly to corn N uptake (Bundy and Malone, 1988), but current recommendations in North Carolina suggest it is not a reliable source of N to be accounted due to high potential for leaching during the wet winter and spring seasons. Therefore, the vast majority of soil-derived N to corn during the growing season would have likely been present in organic form at the time of testing near planting (i.e., 72 to 88% of plant available N in the surface 30 cm of soil). This result emphasizes the need for a soil test that can predict net N mineralization during the growing season.

Determination of plant available N requires considerable analysis time and resources, and suffers from large analytical variations due to relatively small quantity of N from two important forms, i.e., NH_4–N and NO_3–N, which are often determined separately. A more rapid analysis of soil biological activity that is highly related to net N mineralization is the flush of CO_2 following rewetting of dried soil (Franzluebbers et al., 2000; Franzluebbers, 2016). When sampled at depth of 0 to 10 cm, the flush of CO_2 (FCO_2) was associated with relative corn yield in a manner similar to that of plant available N, according to the following:

$$RY = 0.12 + 1.08\,(1 - \exp[-0.00337\,FCO_2]),$$
$$r^2 = 0.60\,(p < 0.001),\ n = 42$$

Sampling soil to lower depths did not substantially change the relationship between relative yield and the flush of CO_2. Coefficient of determination declined to 0.56 at 0- to 20-cm

depth and 0.50 at 0- to 30-cm depth and slopes became more linear than at 0- to 10-cm depth. These results suggest that sampling soil at 0 to 10 cm would be sufficient to characterize soil biological activity for N management in the field.

The flush of CO_2 following rewetting of dried soil is considered an ideal test for soil biological activity, because it (a) relies on dried soil to cause a flush of activity on rewetting, (b) is rapid (3 d of incubation), (c) is biological in nature and not a chemical index, (d) is reliable with low variation among subsamples, and (e) is robust with high correlation in association with strong variations in net N mineralization, cumulative C mineralization, soil microbial biomass C, and basal soil respiration among a diversity of soils and management conditions (Franzluebbers and Stuedemann, 2008; Franzluebbers and Haney, 2017). Soil-test biological activity from each of the 47 test sites at three individual depth increments and two cumulative depths is shown in Table 4. Soil-test biological activity was always greatest at 0- to 10-cm depth and declined with greater depth. Mean and standard deviation among sites were 315 ± 135 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ at a depth of 0 to 10 cm, 103 ± 32 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ at a depth of 10 to 20 cm, 61 ± 22 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ at a depth of 20 to 30 cm, 198 ± 70 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ at a depth of 0 to 20 cm, and 147 ± 48 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ at a depth of 0 to 30 cm. Replicate soil samples within a site had coefficient of variation of 15% across all depths, as compared with the 31 to 43% variation among sites.

Another yield response characteristic that helped to define the extent of soil N availability was yield response at initial dose of N fertilizer application (i.e., the empirically-derived instantaneous yield produced with the first unit of N, as derived from regression at each site). Yield response at initial dose of N would be highest in those trials that had a characteristic nonlinear form as posed in Franzluebbers (2016), that is, the steep slope at low levels of soil N availability and neutral slope at high levels of soil N availability. Yield response at initial dose of N varied considerably among trials for both grain (Table 2) and silage (Table 3). Across sites within a region, yield response at initial dose of N

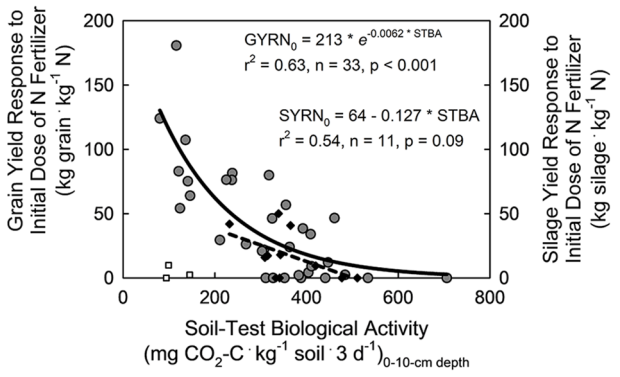

Fig. 3. Association of corn grain (filled circles) and silage (filled diamonds) yield responses to initial dose of N fertilizer (empirical at instantaneous N rate from regression at each site) as affected by soil-test biological activity from the flush of CO_2 following rewetting of dried soil. Three sites (open squares) were removed from the grain regression due to concerns about low yield with adverse weather.

was lowest in the Great Valley for grain (9 ± 10 kg grain kg N^{-1}) and in the Piedmont for silage (17 ± 1 kg silage kg N^{-1}) and highest in the Blue Ridge for grain (105 ± 67 kg grain kg N^{-1}) and in the Great Valley for silage (18 ± 21 kg silage kg N^{-1}). Large variations in estimates of yield response at initial dose of N were due to differences in sufficiency of N at a site, possibly the type of regression model that was selected (i.e., nonlinear and linear), and potentially many other ecosystem factors among sites. Yield response at initial dose of N would have had to be at least 5 kg grain kg N^{-1} and 25 kg silage kg N^{-1} to meet the lowest economic threshold of solvency.

Grain yield response at initial dose of N was significantly negatively associated with level of soil-test biological activity (Fig. 3). The strong negative association of grain yield response at initial dose of N with soil-test biological activity indicates that soil testing could indeed predict whether a site would be responsive to sidedress N or not. In addition, the nonlinear decline in yield response with increasing level of soil-test biological activity as originally hypothesized, suggests soil-test biological activity would be robust enough to not only be able to categorize sites as N-responsive or not, but also describe sites along a continuous gradient of soil N supply. When sampling soil at 0- to 10-cm depth, soil-test biological activity <493 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ indicated progressively greater likelihood of N responsiveness. Sites having soil-test biological activity exceeding this threshold would not be expected to respond to sidedress N application. The threshold level was based on regression and targeting a minimum of 10 kg grain kg N^{-1}. If soil were sampled at 0- to 20- or 0- to 30-cm depth, threshold soil-test biological activity for N responsiveness would have been 305 and 232 mg CO_2–C kg^{-1} $(3\ d)^{-1}$, respectively. The association between silage yield response at initial dose of N and soil-test biological activity was also trending to significance (p = 0.09), and the same general trend occurred as for grain yield response (Fig. 3). Thresholds of soil-test biological activity for N responsiveness in silage trials were relatively similar at 421, 296, and 208 mg CO_2–C kg^{-1} $(3\ d)^{-1}$ when sampled at 0 to 10, 0 to 20, and 0 to 30 cm, respectively.

Lower soil-test biological activity threshold with increasing depth of soil sampling illustrates the importance of surface soil organic matter accumulation, at least in these soils from the southeastern and Mid-Atlantic regions. Whether similar depth relationships exist in other ecoregions remains to be explored. Strong stratification of soil organic matter distribution with depth has been documented in the region with adoption of conservation agricultural systems (Franzluebbers, 2010), and therefore reliance on recent surface-soil organic matter inputs for production of biologically active sources of N is logical.

Relative yield without N fertilizer and yield response to initial dose of N fertilizer clearly described a strong association between soil-test biological activity and responsiveness of corn to additional N. However, these responses did not adequately characterize how much N fertilizer might be needed to achieve economic production. This dataset also offered an opportunity to create a quantitative framework for N recommendations

Table 4. Mean and standard deviation (SD) of soil-test biological activity (i.e., the flush of CO_2 following rewetting of dried soil) as affected by depth of sampling, physiographic region, and location.

	0–10 cm		10–20 cm		20–30 cm		0–20 cm‡		0–30 cm‡	
Field code†	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Soil-test biological activity (mg CO_2–C kg^{-1} [3 d]$^{-1}$)									
Coastal Plain										
LCPS4	121	28	64	5	30	4	91	14	69	10
LCPS5	146	38	94	9	74	17	119	21	103	18
NCCC5	136	30	61	9	24	6	98	18	72	13
NWZC6	237	14	65	6	51	7	146	9	113	9
NWZS6	225	17	72	16	48	6	144	14	110	9
TRS15	99	7	57	4	38	6	78	4	64	4
TRS25	94	13	59	4	39	7	76	5	64	5
TRSD4	145	13	95	9	50	9	123	9	95	12
TRSI4	80	5	63	13	44	16	72	8	63	10
Piedmont										
NLNL5	318	22	73	9	41	9	187	15	136	12
NLNN6	404	50	61	7	27	3	214	20	146	12
NLNP6	409	77	101	31	40	12	242	27	169	19
NLNU5	461	122	75	7	40	6	246	59	171	39
NPJ_6	363	49	79	14	45	14	209	27	150	21
PRSB5	706 [3]§	138	130 [3]	34	64 [3]	5	364 [3]	63	247 [3]	37
PRSB6	534	40	149	20	93	32	302	17	221	23
PRSD5	311	49	76	12	53	21	183	17	137	17
PRSD6	325	37	85	15	61	11	193	24	146	16
PRSS4	141	2	66	1	44	5	102	1	82	2
PRSS5	211	61	70	12	52	11	136	33	106	24
VBFS6	355	57	100	8	58	5	217	25	158	16
VBH35	392	98	125	34	53	14	235	50	165	32
VBH65 (S)	315	47	114	13	50	5	208	27	151	17
VSK_5 (S)	309 [5]	33	101 [5]	6	71 [5]	27	201 [5]	16	155 [5]	16
Great Valley										
VBAA5 (S)	477	88	127	35	70	21	288	51	209	38
VBJ_5	388	14	134	13	67	22	253	2	185	10
VBJ_6	441	39	161	22	98	10	291	13	222	8
VCKA5	411	73	114	11	53	15	248	37	176	21
VDMN6	307	51	99	17	96 [2]	30	198	32	178 [2]	28
VDMS6	303	11	88	14	62 [3]	30	190	3	145 [3]	13
VGE_5	352	57	117	8	53	12	223	25	161	14
VGE_6	383	48	128	18	79	11	243	30	183	23
VHNA5	268 [3]	98	152 [3]	13	74 [3]	7	207 [3]	50	159 [3]	34
VKJ_5 (S)	341	25	107	11	74	25	216	13	164	13
VLK_5 (S)	232	28	63	19	30	12	142	23	102	19
VRD_5	447	41	141	7	97	9	281	16	215	13
VRD_6	327	33	142	20	124	18	229	23	192	18
VRM_5 (S)	343	27	127	34	69	22	229	22	171	17
VRM_6 (S)	365	45	138	26	76 [3]	15	246	30	176 [3]	31
VSD_5 (S)	333	28	99	12	52	9	207	19	152	16
VSD_6	484	13	129	17	102 [3]	23	290	12	219 [3]	17
VSR_5 (S)	339	25	131	30	70	26	228	18	172	20
VSRL6 (S)	420	36	168 [1]	NA	NA	NA	311	NA	NA	NA
VSRU6 (S)	511	32	NA	NA	NA	NA	NA	NA	NA	NA
Blue Ridge										
MHRC5	124	47	81	6	54	22	102	25	86	19
MHRS4	116	17	96	8	77	6	106	6	96	4
MHRS5	238	46	147	15	82	49	191	27	154	34

† Field codes ending with (S) are silage fields.

‡ Soil-test biological activity at 0 to 20 and 0 to 30 cm was calculated from the summation of depth increments calculated from soil C concentration and estimated bulk density using the function: BD = 1.75 exp(−0.0132 SOC).

§ Superscript number at right of mean indicates number of replications deviating from normal of four for all others.

based on soil testing. Calculation of EONR was based on producing as much grain or silage to cover the cost of N fertilizer input. Economic thresholds were determined at low, medium, and high cost-to-value ratios (kilogram of grain or silage per kilogram of N). The N fertilizer required to achieve a low economic threshold of 5 kg grain kg N^{-1} is shown in Table 2 and of 25 kg silage kg N^{-1} is shown in Table 3. Data were expressed absolutely (kg N ha^{-1}) and as a scaled N factor relative to yield level (kg N Mg^{-1} of grain or silage), which could then be used to project requirements for different yield levels. Large variations in estimates were again due to site-specific needs for sidedress N, fit of regression, type of regression equation that was selected (i.e., nonlinear and linear), and potentially many other ecosystem factors among sites. As a summary, EONR was as low as 0 and 40 ± 66 kg N ha^{-1} for silage in the Piedmont and Great Valley regions, respectively, and as high as 177 ± 43 kg N ha^{-1} for grain in the Blue Ridge region.

The EONR was significantly ($p < 0.001$) negatively associated with soil-test biological activity when sampled at 0- to 10-cm depth ($r^2 = 0.45$, $r^2 = 0.42$, and $r^2 = 0.35$ at low, medium, and high threshold levels, respectively). This analysis excluded the same three sites that were of concern in relative yield relationships (TRS15, TRS25, and TRSD4 with low yields due to adverse weather). The negative association of EONR with plant available N at 0- to 10-cm depth was similar, with $r^2 = 0.41$ to 0.43. The slope of EONR as a function of plant available N at 0- to 10-cm depth was −1.19, −1.08, and −0.98 kg N fertilizer kg^{-1} plant available N at low, medium, and high threshold levels, respectively. Value of slopes near −1 suggested that a direct substitution of plant available N could be made for fertilizer N. Therefore, net N mineralization during 24 d of aerobic incubation when sampled at a depth of 0 to 10 cm appears to be a very good projection of seasonal N availability under the environmental conditions during these 3 yr and diversity of sites selected.

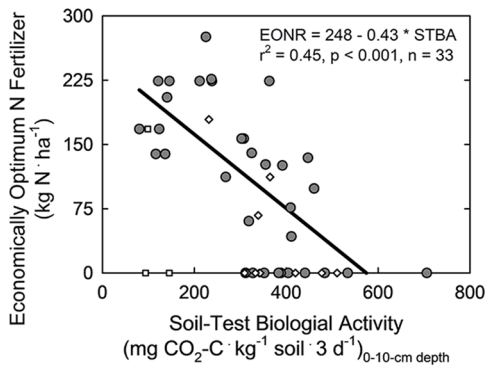

Fig. 4. Association of economically optimum N fertilizer requirement (EONR) to meet low cost-to-value threshold levels for corn grain (filled circles) and silage (open diamonds) production with soil-test biological activity (STBA) when sampled at 0- to 10-cm depth. Note: Three sites (open squares) were excluded due to low yield from adverse weather. Regression and equation shown in graphic is for 33 grain sites only at low cost-to-value threshold (i.e., 5 kg grain kg N^{-1}). Regression equations: EONR at medium cost-to-value threshold = 212 – 0.38 STBA; $r^2 = 0.42$, $p < 0.001$; EONR at high cost-to-value threshold = 169 – 0.32 STBA; $r^2 = 0.35$, $p < 0.001$.

Figure 4 illustrates the relationships of EONR for both grain and silage crops as affected by soil-test biological activity. The low and high cost-to-value threshold levels encompass the range of N fertilizer that might be estimated at any one level of soil-test biological activity. For example, at low soil-test biological activity of 100 mg CO_2–C kg^{-1} $(3\ d)^{-1}$, EONR was between 137 and 205 kg N ha^{-1} to meet high and low cost-to-value thresholds, respectively. At high soil-test biological activity of 500 mg CO_2–C kg^{-1} $(3\ d)^{-1}$, EONR was between 9 and 32 kg N ha^{-1} to meet high and low cost-to-value thresholds, respectively. Current N fertilizer recommendations in North Carolina are based on realistic yield expectations and summarized empirical yield response data from specific soil types (Rajkovich et al., 2015). Recommendations are for 151 to 160 kg N ha^{-1} (0.88 to 0.90 lb N bu^{-1}) in the Coastal Plain, 127 to 174 kg N ha^{-1} (0.86 to 0.93 lb N bu^{-1}) in the Piedmont, and 160 to 255 kg N ha^{-1} (0.66 to 0.93 lb N bu^{-1}) in the Blue Ridge. The mean ± standard deviation of N fertilizer recommended for these regions is 167 ± 37 kg N ha^{-1}, a range that encompassed the low and high threshold levels derived in Fig. 4 associated with low soil-test biological activity. Therefore, farmers utilizing conservation soil management approaches could likely see elevated soil-test biological activity and avail themselves to biologically active soil N from microbial activity to reduce costly N inputs.

To extend these results into a broader N fertilizer recommendation framework based on soil testing, EONR per unit of grain (Table 2) and silage (Table 3) production was calculated. This step provides a scalable function that could be used within reasonable limits of yield levels reported here. Because corn grain yields were reasonably similar among many sites, EONR per unit of grain yield had similar association with plant available N and soil-test biological activity as occurred for EONR on an absolute basis. Strength of association was somewhat greater with soil-test biological activity ($r^2 = 0.27$–0.32, $p < 0.001$) than with plant available N ($r^2 = 0.22$–0.24, $p < 0.01$) as determinant of EONR at low cost-to-value ratio (among different soil sampling depths of 0 to 10, 0 to 20, and 0 to 30 cm). The combination of residual inorganic N at 0- to 30-cm depth (kg ha^{-1}) and 0.241-fold the flush of CO_2 at 0- to 10-cm depth (based on a standard regression in Franzluebbers et al. [2018]) (FINI; flush of CO_2 + inorganic N index) led to even greater association with the economically optimum N fertilizer factor (NF) per unit of grain yield (NF = 24.1 − 0.1138 FINI; $r^2 = 0.34$, $p < 0.001$, $n = 33$). Figure 5 shows the associations of the N factor at three cost-to-value ratios when soil-test biological activity was assessed at depth of 0 to 10 cm and sites were pooled across six nearest values (to more clearly show the trend across the soil-test biological activity gradient). Figure 6 shows similar data, but when soil-test biological activity was assessed at depth of 0 to 20 cm. These relationships illustrate how N fertilizer could be adjusted for site-specific soil conditions based on knowledge of soil-test biological activity. Description of historical management that leads to a change in soil-test biological activity would not necessarily be needed, but there are likely common themes

that can be targeted. In this study, consistent management approaches that likely led to elevated soil-test biological activity in the surface 10 cm of soil were no tillage and application of animal manures. No tillage preserves organic matter from crop residues in surface soil (Franzluebbers, 2010) and animal manures are an important organic matter input to cropping systems (Jenkinson and Powlson, 1976), both of which feed soil microbial communities to sustain higher soil-test biological activity (Haney et al., 2001; Franzluebbers and Stuedemann, 2003).

The following is a summary of how soil-test biological activity correlated ($p \leq 0.01$) with various yield response components. Soil-test biological activity was highly related with relative yield. Coefficient of determination (r^2) for grain trials (n = 36) was 0.33 at depth of 0 to 10 cm and not significant at depths of 10 to 20 and 20 to 30 cm. At depth of 0- to 20- and 0- to 30-cm depths, r^2 was 0.34 and 0.32, respectively. For the 11 silage trials, soil-test biological activity was not significantly related with relative yield at any soil depth. However, when the two datasets were combined (n = 47), coefficients of determination were 0.35, 0.18, 0.06 (not significant), 0.35, and 0.32 at depths of 0 to 10, 10 to 20, 2 to 30, 0 to 20, and 0 to 30 cm, respectively. For grain trials, soil-test biological activity at 0- to 10-cm depth was negatively associated with yield response at initial dose of N fertilizer and with EONR at low cost-to-value threshold. Associations were strong at a depth of 0 to 10 cm and became weaker and insignificant with lower soil depths. However, calculated at depths of 0 to 20 and 0 to 30 cm, correlation coefficients were as strong as at 0- to 10-cm depth only. For silage trials, soil-test biological activity (0–10 and 0–30 cm) was significantly negatively associated only with EONR at low cost-to-value ratio (25 kg silage kg N^{-1}). These associations suggest that soil sampling for soil-test biological activity could be optimized by limiting to 0 to 10 cm, but that sampling deeper to 30 cm could still be a viable option. Sampling too deep risks dilution of high concentrations at the surface with low and variable concentrations with depth.

Several other soil properties were equally well correlated with yield response characteristics as soil-test biological activity (Table 5). Many of these soil properties were significant at 0- to 10-cm depth, but not at lower depths. One notable exception was that of extractable Ca, which was significant at nearly all depths for relative yield and yield response at initial dose of N (Table 5). Another notable observation was the lack of significance of total organic C and N (and P and K concentrations) with yield response characteristics. The lack of association with several traditional soil testing variables corroborates the need to look for alternative soil testing procedures to describe soil biological processes. Soil biological properties of the flush of CO_2, net N mineralization, plant available N, cumulative C mineralization, and basal soil respiration at 0 to 10 cm and often also at 0- to 20- and 0- to 30-cm depths were consistently positively related with relative yield and negatively related with yield response at initial dose of N and EONR at low cost-to-value threshold for corn grain production.

Some soil properties were related with relative silage yield, but few were related with yield response at initial dose of N and EONR at low cost-to-value threshold for silage production (data not shown). The limited number of silage observations would need to be expanded for more robust evaluation. Soil properties positively associated with relative silage yield included total organic C (0–10 and 0–20 cm), residual soil nitrate (0–10, 0–20, and 0–30 cm), residual inorganic N (0–10, 0–20, and 0–30 cm), and cation exchange capacity at 0- to 10-cm depth. Negative associations occurred between relative silage yield and bulk density at 0- to 10-cm depth, suggestive of potential compaction with heavy wagon loads during harvest.

This overview of plant and soil associations illustrates that soil-test biological activity is certainly a reasonable indicator of soil N availability and whether corn responds to additional N inputs. As corroborated here and repeatedly shown in previous greenhouse and laboratory incubation studies (Franzluebbers et

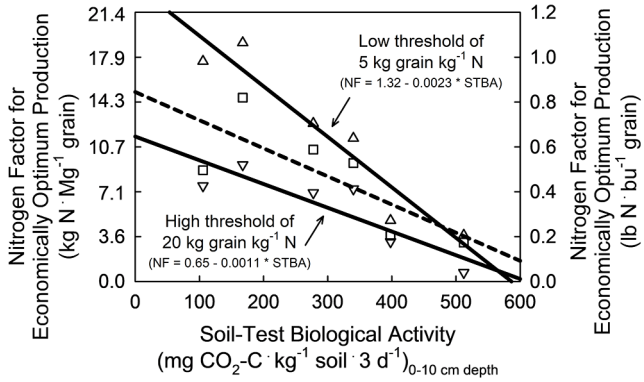

Fig. 5. Association of the N factor for economically optimum production required to meet low, medium, and high cost-to-value threshold levels with soil-test biological activity when sampled at 0- to 10-cm depth. Notes: Data points are means from six neighboring sites when sorted by rank soil-test biological activity (upright triangles are for low threshold, squares are for medium threshold, and inverted triangles are for high threshold). Equations under threshold labels refer to Imperial units on the right vertical axis.

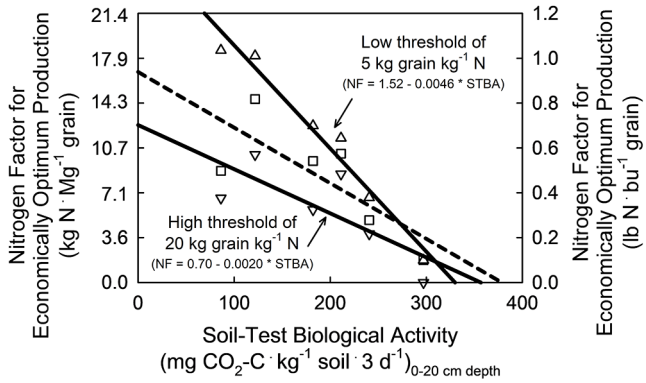

Fig. 6. Association of the N factor for economically optimum production required to meet low, medium, and high cost-to-value threshold levels with soil-test biological activity when sampled at 0- to 20-cm depth. Notes: Data points are means from six neighboring sites when sorted by rank soil-test biological activity (upright triangles are for low threshold, squares are for medium threshold, and inverted triangles are for high threshold). Equations under threshold labels refer to Imperial units on the right vertical axis.

Table 5. Significant ($p \leq 0.01$) correlation coefficients of soil properties associated with corn grain yield response characteristics.

Soil property†	Relative yield					Yield response at initial dose of N					N Required at low cost-to-value threshold				
	Soil depth, cm														
	0–10	10–20	20–30	0–20	0–30	0–10	10–20	20–30	0–20	0–30	0–10	10–20	20–30	0–20	0–30
	Mg Mg^{-1}					kg grain kg N^{-1}					kg N Mg grain^{-1}				
FCO_2	0.57	NS‡	NS	0.58	0.56	−0.55	NS	NS	−0.56	−0.56	−0.52	−0.43	NS	−0.54	−0.52
BSR	0.55	NS	NS	0.55	0.49	−0.56	NS	NS	−0.59	−0.56	−0.49	NS	NS	−0.50	−0.44
CMIN	0.49	NS	NS	0.46	0.41	−0.45	NS	NS	−0.43	NS	−0.45	NS	NS	−0.43	NS
SMBC	0.42	NS	NS	NS	NS	−0.46	NS	NS	NS	NS	−0.48	NS	NS	NS	NS
NMIN	0.51	NS	NS	0.50	0.46	−0.52	NS	NS	−0.53	−0.52	−0.48	NS	NS	−0.47	−0.44
PAN	0.55	NS	NS	0.54	0.50	−0.50	NS	NS	−0.49	−0.45	−0.54	NS	NS	−0.53	−0.50
TOC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TSN	NS	NS	NS	NS	NS	−0.43	NS	NS	NS	NS	NS	NS	NS	NS	NS
POC	NS	NS	NS	NS	NS	−0.41	NS	NS	NS	NS	NS	NS	NS	NS	NS
PON	NS	NS	NS	NS	NS	−0.41	NS	NS	−0.46	−0.44	NS	NS	NS	NS	NS
RSA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RSN	NS	NS	NS	NS	NS	NS	0.47	0.47	NS	NS	−0.40	NS	NS	NS	NS
RIN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	−0.42	NS	NS	−0.44	−0.41
pH	0.49	NS	NS	0.41	NS	−0.49	−0.45	NS	−0.48	−0.43	−0.44	NS	NS	NS	NS
CEC	0.48	NS	NS	0.42	NS	−0.49	NS	NS	−0.48	−0.47	−0.51	NS	NS	−0.46	−0.41
BS	0.60	0.46	NS	0.54	0.46	−0.53	−0.50	NS	−0.52	−0.48	−0.48	NS	NS	−0.42	NS
Acidity	−0.46	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.44	NS	NS
K	NS	NS	NS	NS	NS	−0.47	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ca	0.54	0.43	NS	0.52	0.49	−0.53	−0.50	−0.44	−0.54	−0.53	−0.55	−0.41	NS	−0.51	−0.47
Mg	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mn	0.44	0.45	0.41	0.45	0.44	−0.45	−0.45	NS	−0.46	−0.44	NS	NS	NS	NS	NS
Cu	0.44	NS	NS	NS	NS	−0.42	NS	NS	NS	NS	NS	NS	NS	NS	NS
Zn	NS	NS	NS	NS	NS	−0.41	NS	NS	NS	NS	NS	NS	NS	NS	NS
BD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sand	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Clay	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DM	0.44	NS	NS	0.43	NS	−0.43	NS	NS	−0.41	NS	−0.44	−0.42	NS	−0.44	NS
NU	0.49	NS	NS	0.48	0.42	−0.41	NS	NS	NS	NS	−0.49	NS	NS	−0.50	−0.46

† Abbreviations and units: Acidity (meq dm^{-3}), BD, bulk density (Mg m^{-3}); BS, base saturation (%); BSR, basal soil respiration (mg CO_2–C kg^{-1} d^{-1}); Ca, extractable calcium (mg dm^{-3}); Clay (g kg^{-1}); CEC, cation exchange capacity (meq dm^{-3}); CMIN, cumulative C mineralization (mg CO_2–C kg^{-1})$_{0–24\ d}$; Cu, extractable copper (mg dm^{-3}); DM, dry matter production in greenhouse (g kg^{-1}); FCO_2, flush of CO_2 (mg CO_2–C kg^{-1})$_{0–3\ d}$; K, potassium (mg dm^{-3}); Mg, extractable magnesium (mg dm^{-3}); Mn, extractable manganese (mg dm^{-3}); NMIN, net N mineralization (mg N kg^{-1})$_{0–24\ d}$; NU, nitrogen uptake by greenhouse test plants (mg N kg^{-1}); P, extractable phosphorus (mg dm^{-3}); PAN, plant-available N (mg N kg^{-1})$_{0–24\ d}$; pH (−log[H^+]); POC, particulate organic C (g C kg^{-1}); PON, particulate organic N (g N kg^{-1}); RIN, residual inorganic N (mg N kg^{-1}); RSA, residual soil ammonium (mg NH_4–N kg^{-1}); RSN, residual soil nitrate (mg NO_3–N kg^{-1}); S, extractable S (mg dm^{-3}); Sand (g kg^{-1}); SMBC, soil microbial biomass C (mg C kg^{-1}); TOC, total organic C (g C kg^{-1}); TSN, total soil N (mg N kg^{-1}); Zn, extractable zinc (mg dm^{-3}).

‡ NS, not significant.

al., 2018; Franzluebbers and Pershing, 2018), soil-test biological activity is as good or better than measuring plant available N (based on residual inorganic N + net N mineralization during aerobic incubation for 24 d) for indicating soil N availability. This study also showed the strong dependence on soil sampling depth to derive quantitative relationships for predicting soil N availability and making N fertilizer recommendations using soil-test biological activity. The surface 0 to 10 cm was most enriched in biologically active N and was essential in predictions. Sampling deeper than 10 cm is still legitimate to predict N availability (especially to assess residual inorganic N), but less biologically active soil below 10 cm simply appears to dilute the effect at the surface. At least down to 30 cm, there was no strong deterioration of relationships that were driven by key concentrations in the surface 10 cm.

We focused on soil biological indicators of soil N availability in this evaluation. Other commonly proposed chemical indices of soil N availability may or may not have shown association with yield responses observed in this set of trials. However, the focus on a soil biological indicator that can predict N availability should be considered ideal, especially such an indicator that is also well suited for the conditions of routine soil testing, that is, use of dried soil, rapid analysis time, and calibration with a major crop and one of its primary input variables.

CONCLUSIONS

Corn grain and silage yield responses to sidedress N application were a negative function of soil N availability, i.e., the greater the supply of mineralizable N, the lower the yield response to N application. Supply of N in the surface 30 cm was only partially derived from residual inorganic N and most dominantly

from mineralization of a biologically active portion of soil organic matter, which was especially concentrated in the surface 10 cm of most soils due to history of conservation management. Soil N supply was effectively described as a combination of residual inorganic N (NH_4–N + NO_3–N) and mineralizable N (net N mineralization during 24 d of aerobic incubation at 25°C and 50% water-filled pore space). Soil N supply was positively associated with relative grain and silage yield without sidedress N as compared with non-limiting N supply. Soil N supply was negatively associated with yield response at initial dose of N and economically optimum N fertilizer requirement—a logical association that suggests available soil N reduces the need for N fertilizer inputs. As a rapid, reliable, and robust surrogate of soil N supply, soil-test biological activity from the flush of CO_2 effectively indicated relative yield, yield response to N fertilizer input, and economically optimum N fertilizer requirement. These results clearly suggest that soil testing could be used to adjust N fertilizer recommendations in North Carolina and Virginia. Additional field studies are needed to validate the approach, since large variations among sites were likely caused by a combination of edaphic and environmental factors that influenced whole-plant responses to N acquisition. This field calibration set with a soil biological indicator of N availability will be an important step toward more efficient natural resource utilization in agricultural landscapes.

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