TITLE:

Maize Internode N addition study - in collaboration w/ INRA

AUTHORS:

Zachary L. Rinkes

Dept. of Environmental Sciences

University of Toledo

2801 W. Bancroft St.

Mail Stop 604

Toledo, OH 43606

Isabelle Bertrand

2 INRA, UMR614 FARE

2 esplanade Roland Garros

F-51100 Reims, France

Bilal Ahmad Zafar Amin

2 INRA, UMR614 FARE

2 esplanade Roland Garros

F-51100 Reims, France

A. Stuart Grandy

Dept. of Natural Resources and the Environment

University of New Hampshire

114 James Hall

Durham, NH 03824

(603) 862-1075

<http://pubpages.unh.edu/~asf44/>

Kyle Wickings

Department of Entomology

Cornell University

Geneva, NY 14456

Michael N. Weintraub

Dept. of Environmental Sciences

University of Toledo

2801 W. Bancroft St.

Mail Stop 604

Toledo OH 43606

419.530.2585

<http://www.utoledo.edu/nsm/envsciences/faculty/weintraub.html> [michael.weintraub@utoledo.edu](mailto:michael.weintraub@utoledo.edu)

FUNDING SOURCE AND GRANT NUMBER:

National Science Foundation, Ecosystem Program

Award # 0918718

PHYSICAL LOCATION:

Oak Openings Preserve Metropark, Northwest Ohio

Latitude: 41.55

Longitude: -83.83

INRA Lusignan experimental station, Vienne, France

Latitude: 49.43

Longitude: 0.12

Kitty Todd Nature Preserve, Northwest Ohio

Latitude: 41.62

Longitude: - 83.78

DATA SET OVERVIEW:

This data set contributes information on litter enzyme activities; extractable nitrogen and phosphate; microbial biomass; soil respiration; and litter phenolic chemistry. Data was collected from a 478-day incubation experiment that included three maize genotypes, two different pH soils: 4.9 and 6.7, and two nitrogen addition levels (addition or no-addition). Four replicates of one hundred-twenty litter and soil treatment groups (3 litter types x 2 soils x 2 nitrogen addition levels x 10 harvests) and forty soil-only control groups (2 soils x 2 nitrogen additions x 10 harvests) were harvested for analysis 0, 27, 58, 84, 120, 172, 238, 316, 390, 478 days after incubation start.

BACKGROUND:

<http://www.eeescience.utoledo.edu/Faculty/weintraub/Projects.htm>

SAMPLE COLLECTION:

*Litter and Soil Collection*: Litter at physiological maturity was collected from three different maize genotypes at INRA Lusignan experimental station. Soils were collected from Oak Openings Preserve Metropark and Kitty Todd Nature Preserve in Northwest Ohio. Each soil sample was collected using a metal soil corer with a diameter of 5 cm to a depth of 5 cm from a 10 m² area at both locations. Soils were then taken immediately back to the lab and sieved (2 mm mesh) to remove coarse debris and organic matter and then homogenized by hand. Soil stored in the dark at 20 °C and 45% water holding capacity for 3 months prior to the incubation experiment.

*Incubation*: The incubation experiment included four replicates of forty soil-only control groups (2 soil types x 2 nitrogen addition levels x 10 harvests) and one hundred-twenty litter and soil treatment groups (3 litter types x 2 soil types x 2 nitrogen addition levels x 10 harvests). All replicates were conducted in 0.473 L Mason jars (Ball Pint Wide Mouth Canning Jars, Jarden Corporation). Litter and soil treatment jars received 50 g of soil (dry weight equivalent) at 45% water holding capacity mixed with litter fragments at a rate of 3000 mg C kg⁻¹ soil. Soil-only control jars received only 50 g of soil (dry weight equivalent) at 45% water holding capacity. Jars were kept loosely covered (to minimize water loss, but allow gas exchange) in a dark incubator at 20 °C throughout the 478-day incubation. Nitrogen, in the form of ammonium nitrate, was added to half the litter and soil treatment jars and half of the soil-only control jars in a ratio of 70 mg kg⁻¹ soil. Jars were monitored for soil respiration throughout the incubation (See *Respiration* *Analysis* below). Jars were harvested for litter enzyme activities; extractable nitrogen and phosphate; and litter phenolic chemistry (see below for descriptions). During harvests, litter was removed from soil treatments and litter samples were extracted with 0.5 M potassium sulfate (K₂SO₄). Extractions were performed by placing samples on an orbital shaker at ~120 rpm for 1 hour, and then vacuum filtering the mixture through Millipore APM 15 glass fiber filters. These extracts are labeled as non-fumigated.

Samples were also analyzed for microbial biomass. Microbial biomass carbon (MB-C) and microbial biomass nitrogen (MB-N) were quantified using a modification of the chloroform fumigation-extraction technique (Brookes et al. 1985; Scott-Denton et al. 2006). Following chloroform fumigation, litter was extracted as described above. These extracts are labeled as fumigated. Fumigated and non-fumigated extracts were then frozen until time of analysis.

DATA COLLECTION:

*Litter Nutrients*: Litter nutrients were analyzed using non-fumigated samples. Nitrogen analyses included nitrate and ammonium. Nitrate was analyzed using the method described by Doane & Horwath (2003). Final nitrate values are reported in μg NO3⁻ g-1 dry litter. Ammonium was analyzed using the method described by Rhine *et al*. (1998). Final ammonium values are reported in μg NH4+ g-1 dry litter. Phosphate analysis was conducted using the method described by D’Angelo *et al*. (2001). Final phosphate values are reported in μg PO43- g-1 dry litter.

*Microbial Biomass*: Microbial biomass C and microbial biomass N were analyzed using fumigated and non-fumigated samples. Samples were analyzed for dissolved organic carbon (DOC) and total nitrogen (TN) using a Shimadzu total organic carbon (TOC-VCPN) analyzer equipped with total nitrogen unit (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Microbial biomass was calculated by subtracting non-fumigated sample concentrations of total organic carbon or total nitrogen from fumigated sample concentrations of carbon and nitrogen. Final values are reported in μg C or N g-1 dry litter.

*Litter Enzyme Activities*: Samples were analyzed for hydrolytic and oxidative extracellular enzyme activity. Samples were analyzed for β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), acid phosphatase (PHOS), and phenol oxidase (PHENOX). BG produces glucose from the hydrolysis of cellulose oligomers; NAG, a chitinase, produces N-acetyl glucosamine from the hydrolysis of chitin derived oligomers; PHOS produces phosphate from the hydrolysis of phosphate monoesters such as sugar phosphates; PHENOX is an oxidative enzyme involved in lignin degradation. Hydrolytic enzyme activities (BG, NAG, and PHOS) were analyzed using the fluorometric assay described by Saiya-Cork *et al*. (2002). Oxidative enzymes (PHENOX) were analyzed using the colorimetric assay described by Saiya-Cork *et al*. (2002) and Floch *et al.* 2007.

*Respiration Analysis*: Respiration was analyzed using sodium hydroxide (NaOH) traps and the barium chloride (BaCl₂)/hydrochloric acid (HCl) titration method described by Snyder and Trofymow (1984). Final respiration values are reported as the amount (mg) of litter C respired over specific time intervals.

*Phenolic Chemistry Analysis*: Litter and soil treatment jars analyzed for phenolic chemistry 0, 120, and 478 days after incubation start. Samples were oxidized with cupric oxide following procedures described by Hedges and Ertel (1982) and Kogel (1986).

NAMING CONVENTIONS:

The following refer to headers or terms used in the data spreadsheets:

*General definitions:*

day: day of harvest

genotype: 3 maize genotypes used in study (F2, F2bm1, F292bm3)

pH: pH 4.9 (acidic) or pH 6.7 (neutral) treatment

N: nitrogen treatments with added nitrogen (+), no added nitrogen (-)

replicate: n=4 or n=2 (CuO analyses)

blank cells=no data available; cell value = 0 means no activity

*CuO Analyses*

N: nitrogen treatments with added nitrogen (+), no added nitrogen (-)

V: vanillyl-type monomers calculated as the sum of the concentrations of vanillin, acetovanillone, and vanilic acid in umol g Org C -1

S: syringyl-type monomers calculated as the sum of the concentrations of syringic acid, syringaldehyde, and acetosyringone in umol g Org C-1

Cn: Cinnamyl-type monomers calculated as the sum of the concentrations of ferulic and p-coumaric acids in umol g Org C-1

VSCn\_Total: The sum of V, S, and Cn type phenols released from lignin macromolecules used to approximate the amount of lignin phenols in umol g Org C-1

SV: ratio of S-type monomers to V-type monomers

(Ac/Al)V: acid to aldehyde ratio of V-type monomers

(Ac/Al)S: acid to aldehyde ratio of S-type monomers

*Harvests:*

NAG: N-acetyl-beta-glucosaminidase activity, nmol hr-1 g-1 litter (fluorescent enzyme assay protocol)

BG: Beta glucosidase activity, nmol hr-1 g-1 litter (fluorescent enzyme assay protocol)

PHOS: Phosphatase activity, nmol hr-1 g-1 litter (fluorescent enzyme assay protocol)

PHENOX: Phenol oxidase activity (ABTS as substrate). µmol g-1 litter hr-1 (colorimetric microplate protocol)

MBC: microbial biomass C, ug C g-1 litter, measured in K2SO4 extract on Shimadzu analyzer

TOC: Total dissolved organic C, ug C g-1 litter, measured in K2SO4 extract on Shimadzu analyzer

MBN: microbial biomass N, ug N g-1 litter, measured in K2SO4 extract on Shimadzu analyzer

TN: Total dissolved nitrogen, ug N g-1 litter, measured in K2SO4 extract on Shimadzu analyzer

NH4: µg NH4-N g-1 litter from 0.5M K2SO4 extractions using microplate assay; no data available for final 2 harvests

NO3: µg NO3-N g-1 litter from 0.5M K2SO4 extractions using microplate assay; no data available for final 2 harvests

PO4: µg PO4-P g-1 litter from 0.5M K2SO4 extractions using microplate assay

*Respiration*

day traps harvested: days after trap placement that the trap was collected for analysis

C trapped: the amount of litter C trapped (mg) between each time interval in days (NaOH traps were changed on each harvest day). Each treatment started with 150mg of litter C - all values in rows 1 through 529 are corrected for soil only controls.

LINKS:

<http://www.eeescience.utoledo.edu/Faculty/weintraub/Projects.htm>

REFERENCES:

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation

and the release of soil nitrogen: a rapid direct extraction method to measure

microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837e842.

D'Angelo, E., Crutchfield, J., Vandiviere, M., 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. J. Environ. Qual. 30, 2206-2209.

Doane, T.A., Horwath, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical Letters 36, 2713-2722.

Hedges JI, Ertel JR (1982) Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Anal Chem 54:174-178

Kogel I (1986) Estimation and decomposition pattern of the lignin component in forest humus layers. Microbial Ecol 18:589-594

Rhine, E.D., Sims, G.K., Mulvaney, R.L., Pratt, E.J., 1998. Improving the berthelot reaction for determining ammonium in soil extracts and water. Soil Sci Soc Am J 62, 473-480

Saiya-Cork KR, Sinsabaugh RL, Zak DR, 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34: 1309e1315.

Scott-Denton, L.E., Rosenstiel, T.N., Monson, R.K., 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology 12, 205-216.