Optimizing arsenic phytoremediation:
Effects of fertilizer on brake fern arsenic uptake and biomass production in heterogeneous field conditions

S. MATZEN*, A. OLSON, C. PALLUD

Environmental Science, Policy and Management, University of California, Berkeley, CA (*smatzen@berkeley.edu)

INTRODUCTION

- **Arsenic (As) soil contamination is widespread,** a result of mining activities, coal combustion, pesticide use, and irrigated agriculture.
- **Phytoremediation with the As-hyperaccumulating fern *Pteris vittata* L. ([1], Fig. 1) has emerged as an *in situ* technology to remediate soils with shallow As contamination.** While the mechanisms of As uptake and accumulation in the fern have received attention in numerous greenhouse and hydroponic experiments, only a few studies have investigated the fern's performance under field conditions. Understanding field performance under complex and heterogeneous conditions is crucial to developing successful *in situ* remediation methods.
- **We designed this project in response to community interest** in the fern's potential to remove As from the site of a proposed neighborhood orchard.

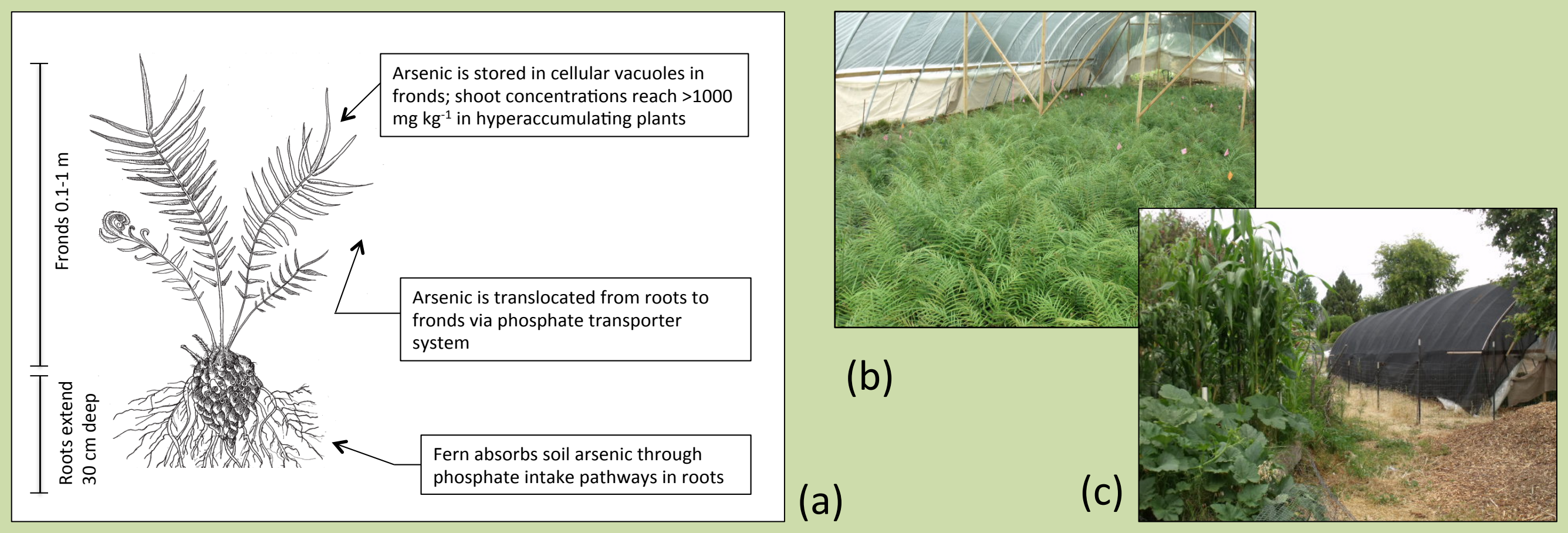

Figure 1. Arsenic hyperaccumulation in *Pteris vittata* L. (a), native to southeast China. At our field site, ferns grow in a hoop house (b); fronds are harvested to remove As from soil. Neighbors are already using the lot for agriculture adjacent to our hoop house (c).

OBJECTIVES

- To understand the role of fertilization in enhancing As phytoremediation efficiency, and thus develop more efficient methods for *in situ* arsenic remediation
- Specifically, to determine the effects of organic and inorganic fertilization on *P. vittata* frond biomass, As uptake rate by *P. vittata,* and on cumulative As removal from soil

METHODS

Figure 2. Site location.

Field site: An abandoned railroad right-of-way with heterogeneous soil texture and bulk density (Fig. 7) mildly contaminated with As (85.5±9.3 mg kg^{-1}), located in Berkeley (CA) (Fig. 2), characterized by a Mediterranean climate.

Experiment design: A 24m x 6m plot was tilled and limed (3,000 kg $CaCO_3$ ha^{-1}) before 1,600 *P. vittata* ferns were planted in February 2013 at 30 cm spacing. Five treatments were applied to separate plots at standard agricultural rates (Fig. 3). For comparison, we established a control plot with no treatments applied to ferns.

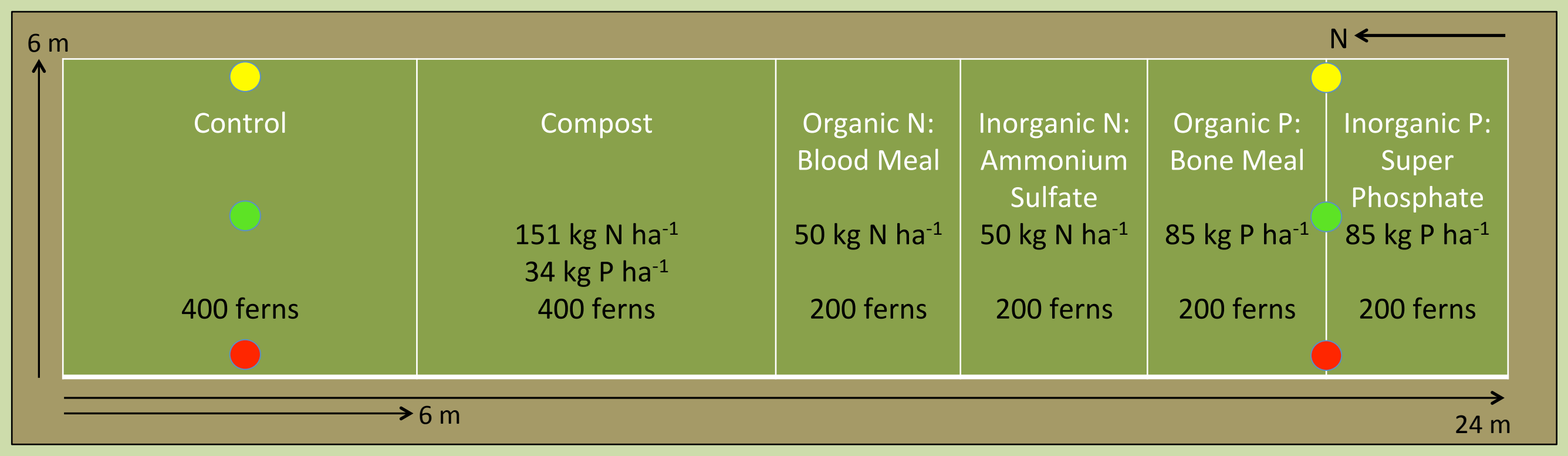

Figure 3. Field experiment schematic. Colored circles show location of soil texture cores.

Data analysis: After 8 months of growth, all mature and senescing fronds were harvested separately. Ten percent of ferns in each treatment group were randomly selected to comprise our representative sample. Individual ferns were kept separate throughout analysis. Arsenic concentrations were analyzed in fern and soil samples using ICP-AES (EPA Methods 3050B and 6010B). Using one-way analysis of variance, significant differences in treatment effects were determined using Duncan's multiple range test, at $p<0.05$.

To determine soil texture, cores were taken along E-W transects at N and S ends of the plot, at 0-15 cm and 15-30 cm depths. Bulk density cores were taken separately from E and W sides of plot in triplicate.

REFERENCES

[1] Ma, L. *et al*. (2001) *Nature* **409**, 579.

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RESULTS

Result 1: Biomass production

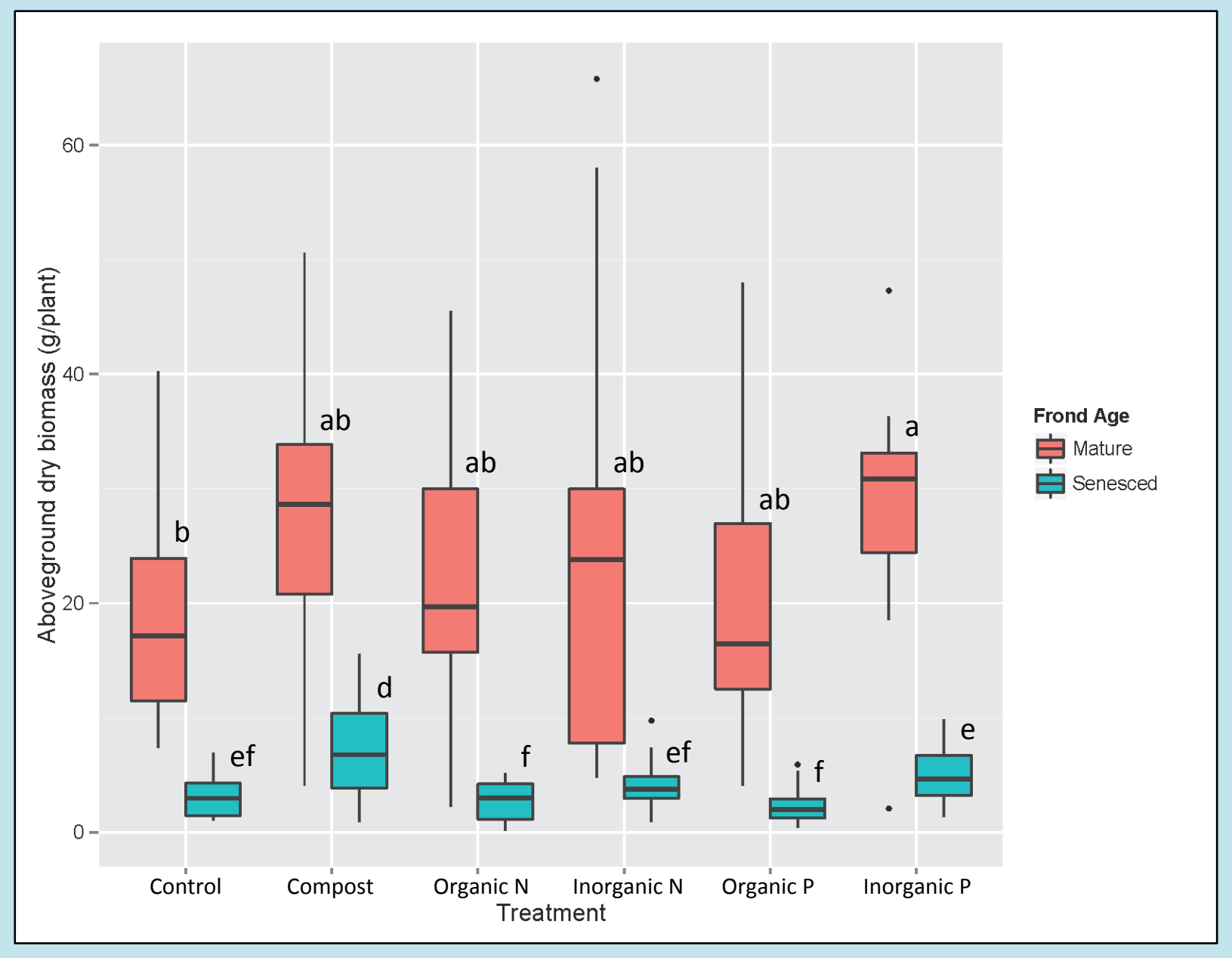

Figure 4. Effects of soil treatment on harvested aboveground dry biomass. n=28 for Control and Compost; n=14 for other treatments. Means with the same letters are not significantly different at $p<0.05$.

Result 2: Arsenic uptake in aboveground biomass

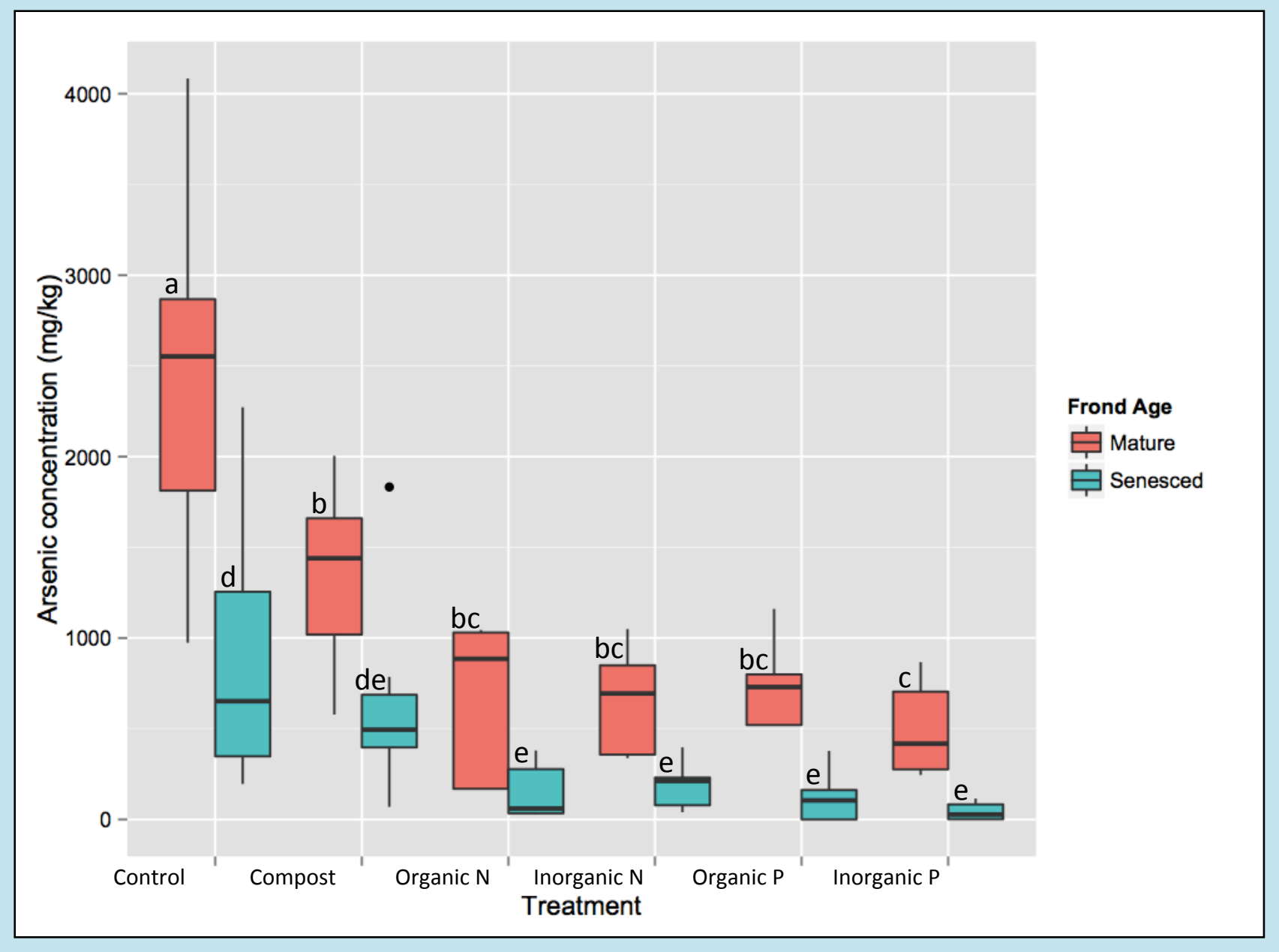

Figure 5. Effects of soil treatment on arsenic concentration in harvested biomass. n=10 for Control and Compost; n=5 for other treatments. Means with the same letters are not significantly different at $p<0.05$.

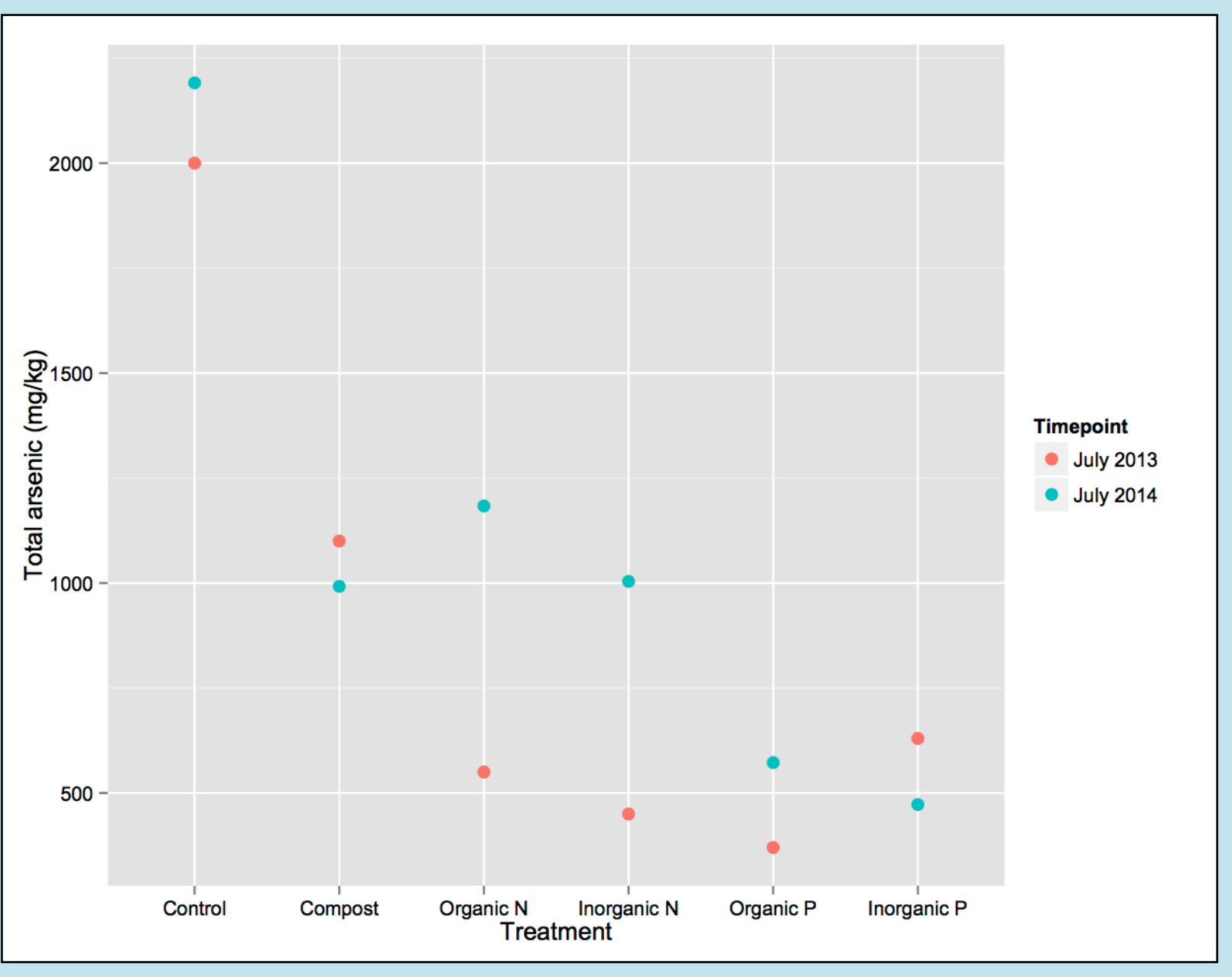

Figure 6. As uptake in pinnae sampled mid-season. Each sample is a composite of 200 pinnae collected from individual plants.

Result 3: Arsenic removal from soil

Table 2. Efficiency of different treatments for arsenic removal and estimated remediation time to reach an assumed regulatory standard of 10 mg kg^{-1}.

Soil treatment	Initial $[As]_{soil}$ ($mg\ kg^{-1}$)	As uptake per plant biomass ($mg\ g^{-1}$)	Total As removed ($kg\ ha^{-1}\ y^{-1}$)	Years to remediate our study site
Control	85.5	2.7	7.8	40
Compost		1.2	6.9	46
Organic N		0.61	2.5	127
Inorganic N		0.58	2.8	113
Organic P		0.69	2.6	122
Inorganic P		0.41	2.3	138

SITE CHARACTERIZATION

Soil texture heterogeneity

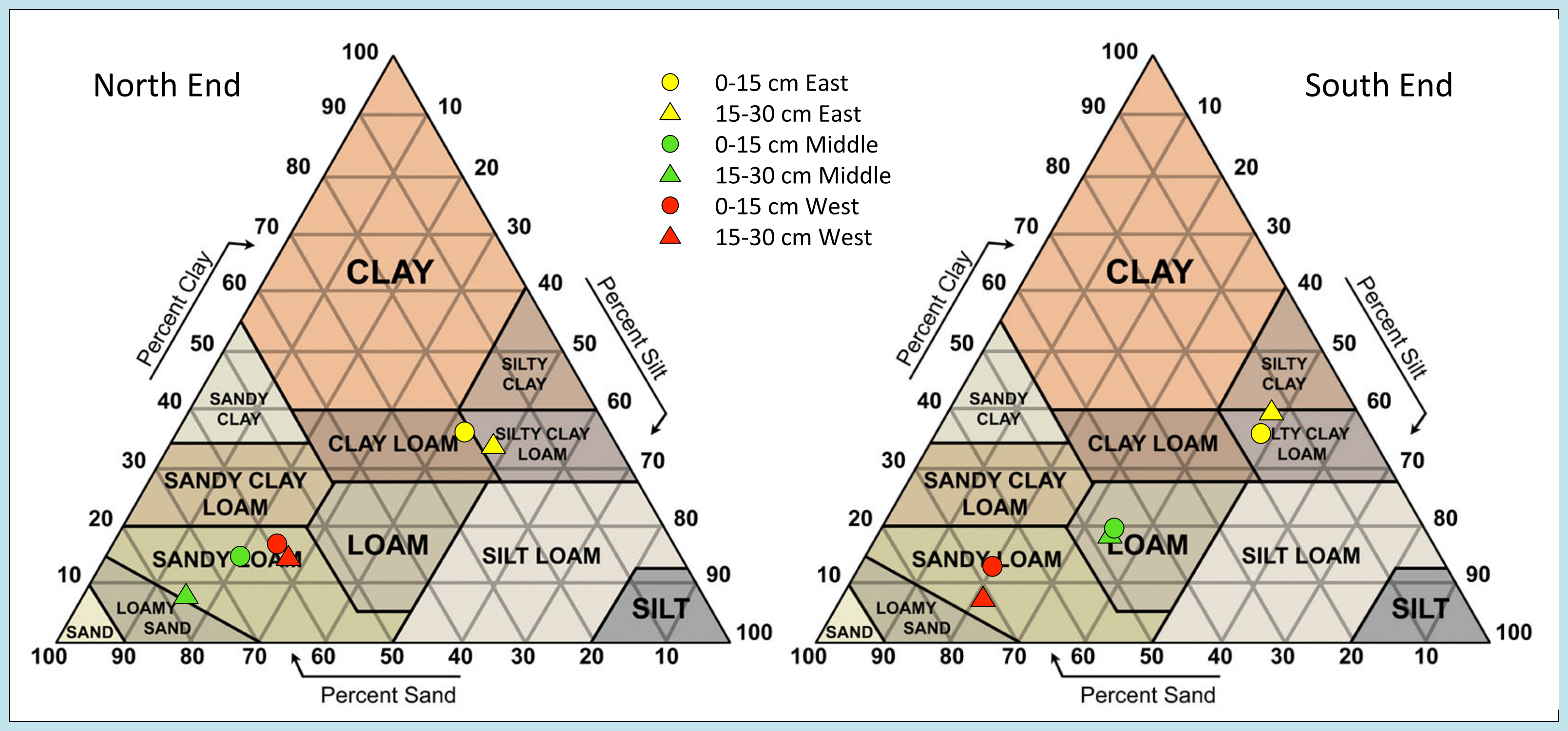

Figure 7. Soil texture gradient across experimental plot at two depths; results are averages of two replicates. East (clayey) side bulk density is 1.06±0.07 g/cm^3; west (sandy) side bulk density is 1.20±0.06 g/cm^3. Sand is most likely due to railroad grade fill.

Soil pH, carbon content and nitrogen content

Table 2. Soil pH, C, and N contents for experimental plots at two depths; initial results are compared to values after 8 months of growth at first harvest. Reported values are averages of 3 replicates, except for Control – Ferns and Compost values, averages of 6 replicates. *1 composite sample of 3 replicates

	Depth (cm)	**Initial Soil**	**After 8 months of growth**						
Treatment		Whole Plot	No Fern Control	Control Ferns	Compost	Organic N	Inorganic N	Organic P	Inorganic P
pH	0-15	6.9	6.6	7.3	7.1	6.7	6.1	6.6	6.7
	15-30	6.9*	7.0	7.5	7.2	6.6	6.6	6.8	7.0
%C	0-15	4.8	2.5	2.6	3.5	3.8	4.0	3.2	2.8
	15-30	4.0	1.1	1.3	2.0	2.3	2.2	2.7	1.5
%N	0-15	0.38	0.16	0.17	0.23	0.25	0.25	0.21	0.18
	15-30	0.29	0.08	0.09	0.13	0.13	0.13	0.15	0.09
C/N	0-15	12.7	16.1	15.4	15.2	15.6	16.2	15.0	15.8
	15-30	13.8	14.2	15.0	15.5	18.1	16.5	17.1	16.1

CONCLUSIONS

- **Control ferns were most effective at removing As, followed by compost-amended ferns.** Improving As phytoremediation efficiency depends on first increasing As uptake in the fern, followed by increasing aboveground biomass.
- All other treatments (inorganic and organic N, inorganic and organic P) decreased As accumulation by a factor of 2 to 3, compared to control and compost-amended ferns. Ferns in N and P plots did not concentrate As above the hyperaccumulation threshold of 1,000 mg As kg^{-1} plant. ***P. vittata* does not behave as an As hyperaccumulator under some field conditions.**
- Senesced fronds lost As, possibly due to As leaching into soil at fern base. Timing harvests to avoid senescence could improve As removal by 12%.
- The eastern third of the plot has 36% clay, whereas the western two-thirds have 7-20% clay. Heterogeneous soil texture could increase intra-plot variability in As content, As phytoavailability, interactions of fertilizers with As, and consequently the fern's ability to uptake As.
- Preliminary July 2014 data suggest As uptake in ferns amended with organic and inorganic N is higher in Year 2 than in Year 1 by a factor of 2:1.

FUTURE RESEARCH

- Investigate effects of mycorrhizal fungi and soil texture on As accumulation in the fern.