Relationship of Available Nutrients to Chlorophyll Content of Two Ferns In Arcata Community Forest Humboldt County, CA

Ashley Dickinson, James Gomez, Jo Jindrich, Sara Larson, Katy Watson

Abstract

Redwood forests are important ecosystems consisting of many plants and animals. Dominated by many conifers, these forests also have many plants from the phylum Tracheophyta, also known as ferns. In a case study set in the Arcata Community Forest of Humboldt County, California, we focused on two species within this phyla, western sword fern (*Polystichum munitum*) and western bracken fern (*Pterdium aquilinum*) and compared their environmental and physiological elements. The goals of this study were to observe how these two species assimilate nutrients given similar environmental conditions, to compare each species' chlorophyll production levels, and compare various nutrients available in soil to ultimately see if there was any correlation. Differences in chlorophyll production and nitrogen assimilation were observable between the two species. Contrary to what was expected, however, the results of our study did not reveal distinct correlations between chlorophyll content and iron available in soil. Potassium and calcium levels in topsoil were loosely correlated with chlorophyll concentration of both species.

Introduction

During the light dependent reactions of photosynthesis, photophosphorylation occurs within the thylakoid membrane of chloroplasts found in plant cells. This process is responsible for creating the energy necessary to fuel the light independent reactions of photosynthesis and ultimately the production of sugars that keep not only the plant, but any organism dependent on that plant alive (Allen et al, 2011). Photophosphorylation depends heavily on chlorophyll pigments to capture energy from light. These pigments are so important to the functioning of the plant that any nutrient deficiency that leads to a decrease in chlorophyll will likely result in death of the plant if not rectified. Understanding the role of the nutrients involved in chlorophyll production and photophosphorylation, and how they are obtained from the environment is essential for understanding the health of habitats and their ability to support life given any environmental stressors.

Beyond carbon and oxygen, the essential nutrients required by plants are taken up from the soil for most species. A primary component of all proteins, nitrogen is present in the many complexes involved in the photophosphorylation processes. It also forms the basis of the porphyrin ring center of the chlorophyll molecule (Schmidhalter 2005). In fact, it has been found to be so strongly correlated with chlorophyll concentrations that remote, handheld chlorophyll meters are utilized in the agriculture industry to assess nitrogen deficiency in crops in order to determine fertilizer application

Many of the processes involved in photosynthesis are regulated by potassium ions including CO_2 uptake via the opening and closing of stomata and activation of enzymes that lead to production of Adenosine Triphosphate (ATP) (Dreyer 2017). Specific to chlorophyll production, Tränkner et al (2018) found that potassium is involved directly in the formation of grana and lamellae, which create the structure in which the light dependent reactions of photosynthesis take place and the boundary that facilitates the proton gradient that drives ATP synthesis. The results of their research found a deficiency in potassium resulted in a reduced number of grana and lamellae and poorly formed lamellar membranes.

The vast majority of iron found in leaves is actually located in the chloroplasts (Abadia 1986). Iron is an integral component of both photosystem I and II, cytochrome B_6/f, and ferredoxin, which are the main complexes in the photophosphorylation processes. Interestingly, one study focused on sugar beet plants found that three-fifths of the total iron content in leaves undergoing rapid growth were found in the thylakoid membrane, and one-fifth was found in the stroma, leaving only one-fifth remaining that was outside the chloroplasts (Terry and Low 1982). The results of that study also showed that leaf chlorophyll content was correlated to leaf content of chloroplast iron.

While calcium is not an actual component of chlorophyll or the complexes involved in photosynthesis, it plays an important role in cell division and growth of plant biomass (Nathan 2017). This is not only true above ground, but also in the below ground structures as well. The formation of adequate root systems provides the opportunity for the plant to efficiently take up other essential nutrients from the soil. To enhance this effect, calcium exudates from the plant roots are also involved in signaling arbuscular mycorrhizal fungi to colonize root tissues, which effectively increases root surfaces area and allows for greater nutrient and water uptake by the plant (Leitão et al, 2019). It would stand to reason that a deficiency in calcium in the soil could indirectly decrease the chlorophyll production by limiting other essential nutrients from reaching plant photosynthesizing tissues.

The study sites for this investigation were found within the Arcata Community Forest which is comprised of mostly second growth coastal redwood (*Sequoia sempervirens)* forest. It includes approximately 2,311 acres of land in Humboldt County, California (Fig. 1). It was extensively logged during the early part of the 19th century and then used primarily for grazing. In 1955, the City of Arcata took ownership of these properties making it the first municipally owned forest in California (Van Kirk 1985). The most western boundary of the forest borders the Humboldt State University property, which provided an ideal opportunity for our observational study. The forest in this area is dominated by coastal redwood, western sword fern, and redwood sorrel (*Oxalis oregana).*

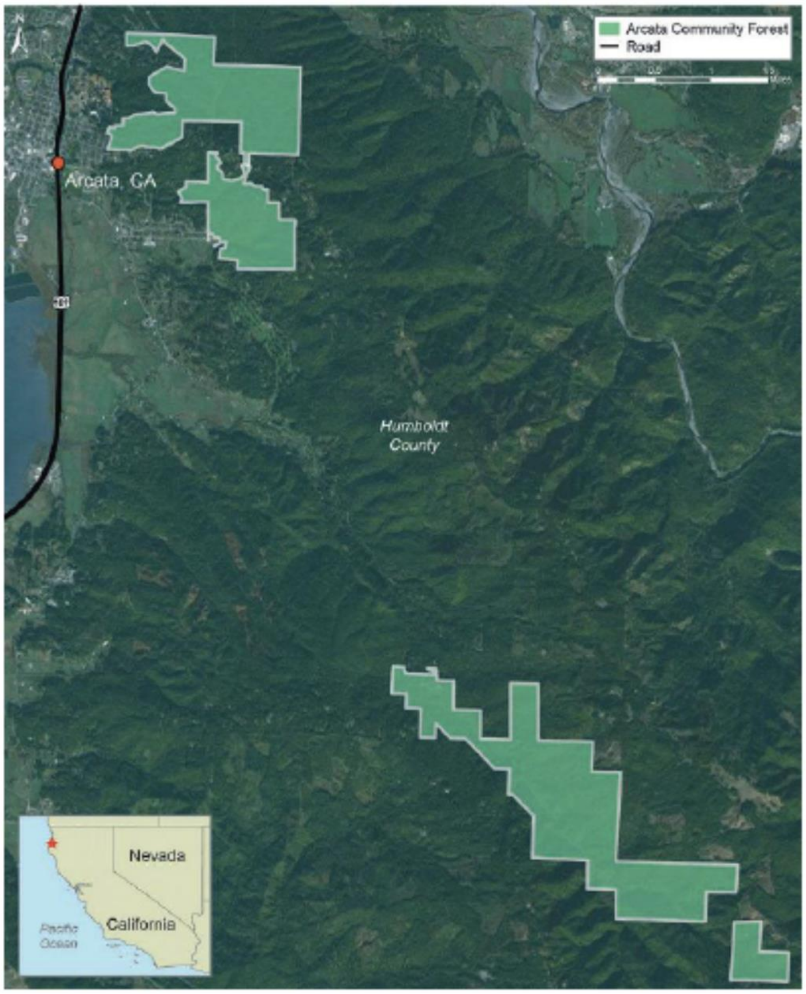


Figure 1. Map of Arcata Community Forest, Humboldt Co, California

Due to its dominance in the understory of the Arcata Community Forest, western sword fern was chosen as the first study species. It is an evergreen fern with a stout erect rhizome that sprouts in a dense cluster. The thin and leathery blades are 1-pinnate and can be between 50 – 200 cm in length. The species can be found in mesic coniferous and moist evergreen forests found at low to mid elevation (<1600 m)(Baldwin et al, 2012). They are best adapted to low light conditions. The distribution of western sword fern is limited to the western portion of North America and primarily the Pacific Northwest (Soltis et al, 1987). Within this region they are very common and are often the dominant understory species in these forested habitats

The second species analyzed in this study, western bracken fern, is described as a deciduous, rhizomatous herb. Frond length is generally between 25 – 250 cm, and has a wide, triangular blade that is 3-pinnate (Baldwin et al, 2012). The rhizomes of this species are generally deep and extensive, sometimes forming dense stands. It is very tolerant of a wide variety of soils, arid conditions, and intense direct sun (Daniels 1985). It also reproduces vigorously and can regenerate easily following fire. These attributes have resulted in a worldwide distribution of the species, so much so that it is often considered to be invasive. It has been designated one of the five most common weed species in the world (Vetter 2009). This aggressive colonization strategy could indicate that this species is particularly successful at obtaining and utilizing nutrients efficiently.

The goal of this research was to explore the relationship between the amounts of nitrogen, potassium, calcium and iron in the soil to photosynthetic function of the two ferns. Specifically, the questions addressed were:

1. What effects do soil nutrient levels have on chlorophyll production within the two species?
2. Do the different species utilize these nutrients differently?

We hypothesized that there would be a positive correlation between chlorophyll concentration and each of the nutrient levels identified based on the direct or indirect involvement of each of these nutrients in chlorophyll production or the photosynthetic pathways. An observable difference in the utilization of these nutrients by the two different fern species was also expected based on differences in the species adaptability to environmental variability. The results of this case study will add to the broader knowledge of plant-soil dynamics. A better understanding of specific plant-soil interactions can also help to inform management decisions surrounding the conservation of plant communities and the management of invasive species.

Methods

Site Selection

The Arcata Community forest was chosen as our collection site based on its close proximity to campus. In order to limit any outlying factors, we chose sites that had a minimum of a 50-meter distance between each other that contained both western bracken fern and western sword fern species. The locations of each site are as follows:

Site 1: (40.8749000, -124.0731000)
Site 2: (40.8754463, -124.0729610)
Site 3: (40.8751360, -124.0737770)

We used our samples for chlorophyll extraction and stored some in the drying oven to use for kjeldahl organic nitrogen assessment.

Chlorophyll Extraction

We ran a chlorophyll extraction analysis with our two species using fresh fern samples and an 80% acetone solution. After extracting all the chlorophyll through a filtration system, the solutions were read through a Spec 20 to calculate the percent transmittance, which could then be converted into the amount of chlorophyll by a series of calculations through Arnon's protocol (Arnon 1948).

Plant Nitrogen Analysis

We ran three replicates of each location site, as well as two positives and two blanks, for a total of 13 reactions. We oxidized the fern sample compounds using strong and hot sulfuric acid and ran them through the digester. The carbon in these samples converts into carbon dioxide, while the hydrogen is converted into water. Nitrogen is converted into ammonium ions that dissolve into the oxidizing solution, which can be converted into ammonium gas later.

A distillation experiment was conducted by adding a strong base (OH^-) to the digestion product to convert NH_4 to NH_3. We use titrations to determine the amount of ammonia in the receiving solution. We can use the amount of ammonium ion to calculate the amount of nitrogen in a sample to compare the two separate ferns from each other. The data collected was used by the protocol from the Kjeldahl organic nitrogen assay lab (Kjeldahl et al, 1883).

Soil Nutrient Analysis

We used falcon tubes to collect soil samples at each collection site. We collected topsoil and dug approximately one foot deep to collect subsoil samples, for a total of six collections. They were stored in a drying oven to be used for our soil nutrient analysis.

The quantification of Iron, Phosphorous, Calcium, and Lead were accomplished by isolating extractable minerals from soil samples taken from each site. Extractable minerals were obtained from the incubation of soil samples in a dilute double acid solution (0.05N HCl + 0.025N H2SO4) and then filtered using fine filter paper. These samples were diluted in a way that the atomic absorption spectrophotometer (AAS) could get ideal reads and then normalized to one another to parts per million. Samples were read using the AAS (Perkin Elmer AAS 400) and calibrated using previously established protocol (Lu 2019, Unpublished) that was used in the lab.

Results

Chlorophyll Analysis

Absorbance values of chlorophyll a and b within the two fern species are recorded along with mass of sample and filtrate volume that we used to find the chlorophyll concentrations (Tables 1 and 2) . The figure below shows a comparison of chlorophyll a concentration between both fern species. Site 3 had the highest amount of chlorophyll overall while western bracken fern typically had higher chlorophyll concentrations than the western sword fern. The chlorophyll b concentrations were comparable to the chlorophyll a level with site 3 and western sword ferns being the overall highest (Fig. 3). When looking at the total chlorophyll levels, the trend seen before with chlorophyll a and b concentrations is still present. Western bracken fern had a higher total chlorophyll concentration at every site (Fig. 4). After running a one-way ANOVA, there was no statistical significance between the chlorophyll concentrations of the two fern species studied (P-value > 0.05).

Table 1: Chlorophyll A & B absorbance values for bracken fern.

	Chl A (663nm)	Chl B (645nm)	Mass of sample (g)	Filtered volume (mL)
Site 1	0.569	0.432	0.5088	20.0
Site 2	0.523	0.367	0.5226	17.5
Site 3	0.415	0.337	0.5050	47

Table 2: Chlorophyll A & B absorbance values for western sword fern

	Chl A (663nm)	Chl B (645nm)	Mass of sample (g)	Filtered volume (mL)
Site 1	0.886	0.602	0.502	54
Site 2	0.824	0.569	0.511	52
Site 3	0.201	0.125	0.524	68

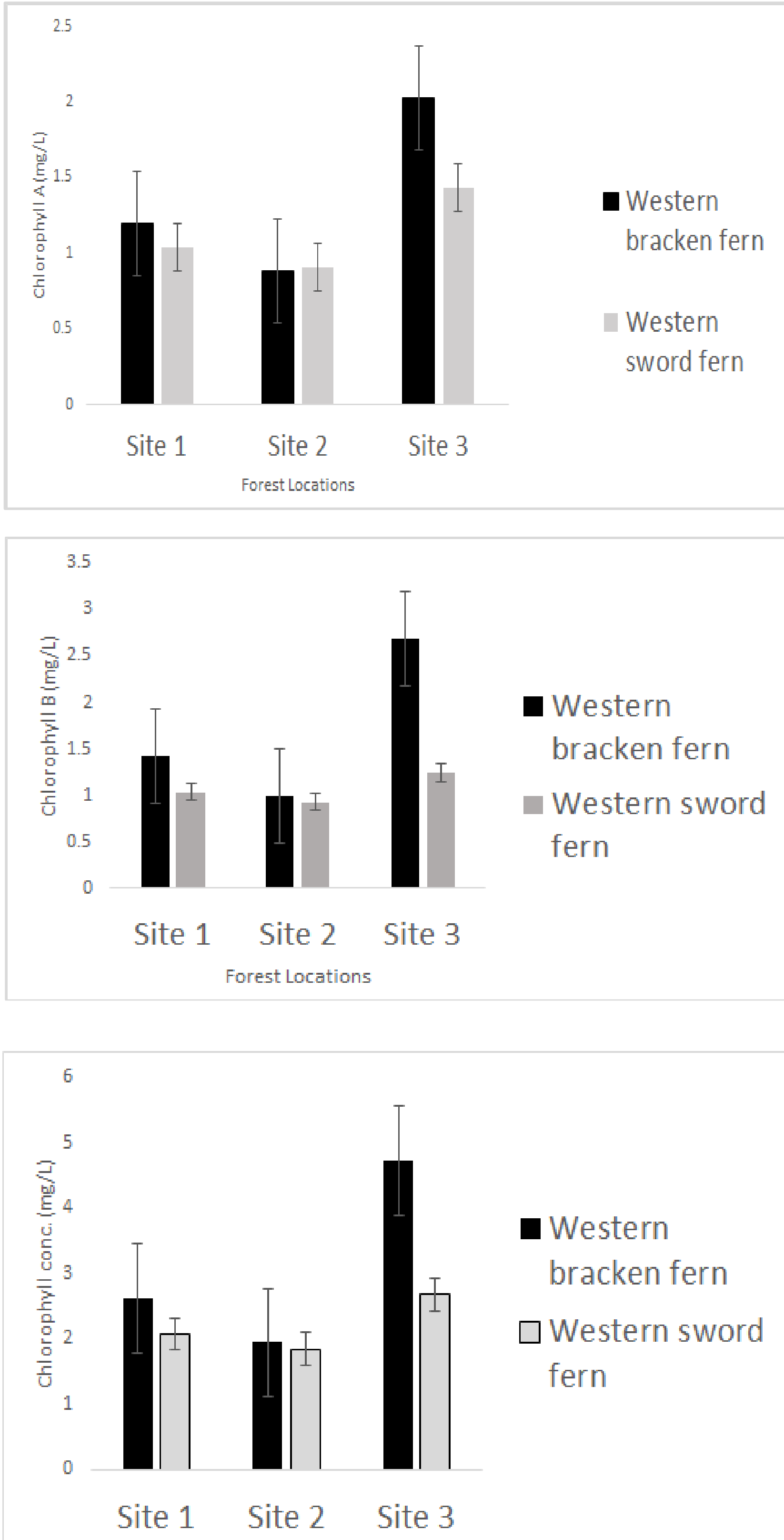


Figure 4. Comparison of (a) chlorophyll a, (b) chlorophyll b, and (c) total chlorophyll concentrations between fern species at each site

Plant Nitrogen Analysis
The results from the Kjeldahl nitrogen assay showed that there were similar levels of nitrogen present in the fronds at each site (Fig. 5). The western bracken fern had the highest amount of nitrogen at each site, similar to chlorophyll concentrations previously stated. There was no statistical significance between the sites or ferns from running a one-way ANOVA (P-value > 0.05).

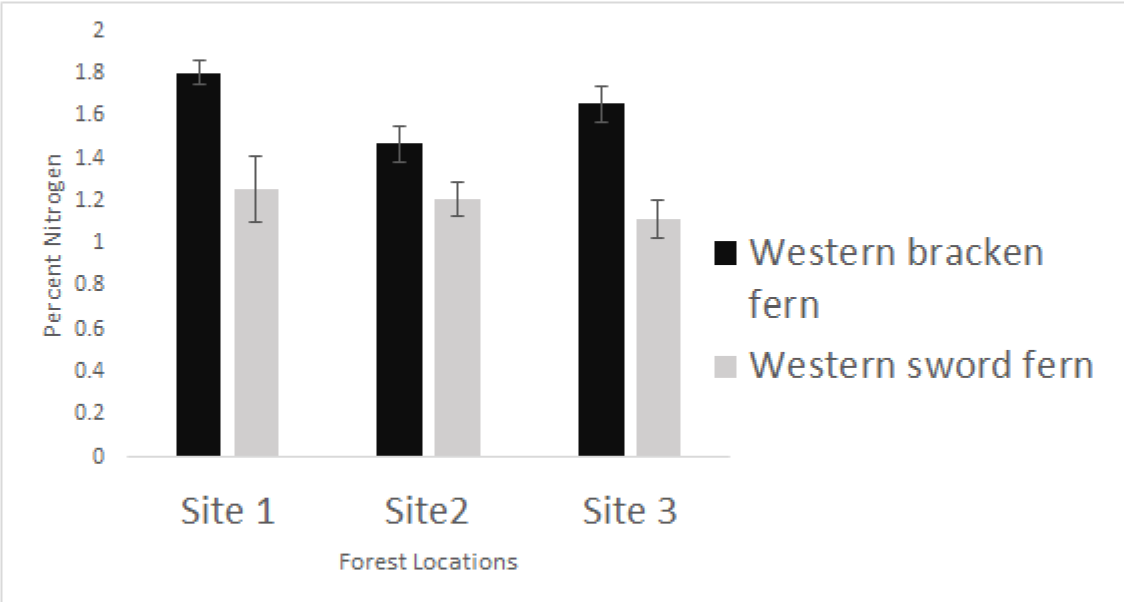


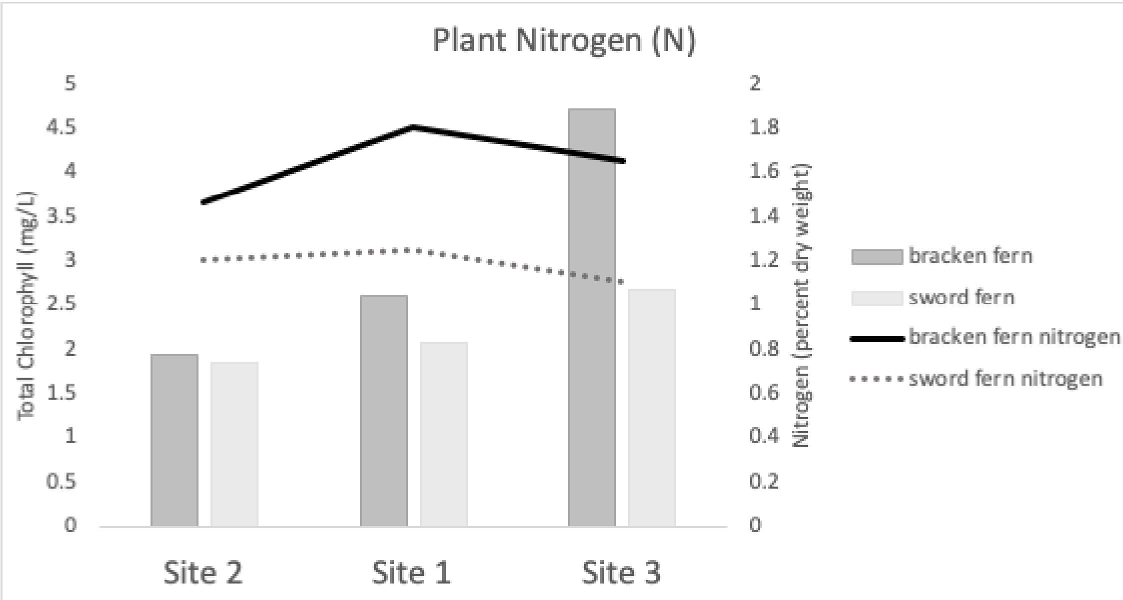
Figure 5. Comparison of nitrogen content between western bracken fern and western sword fern

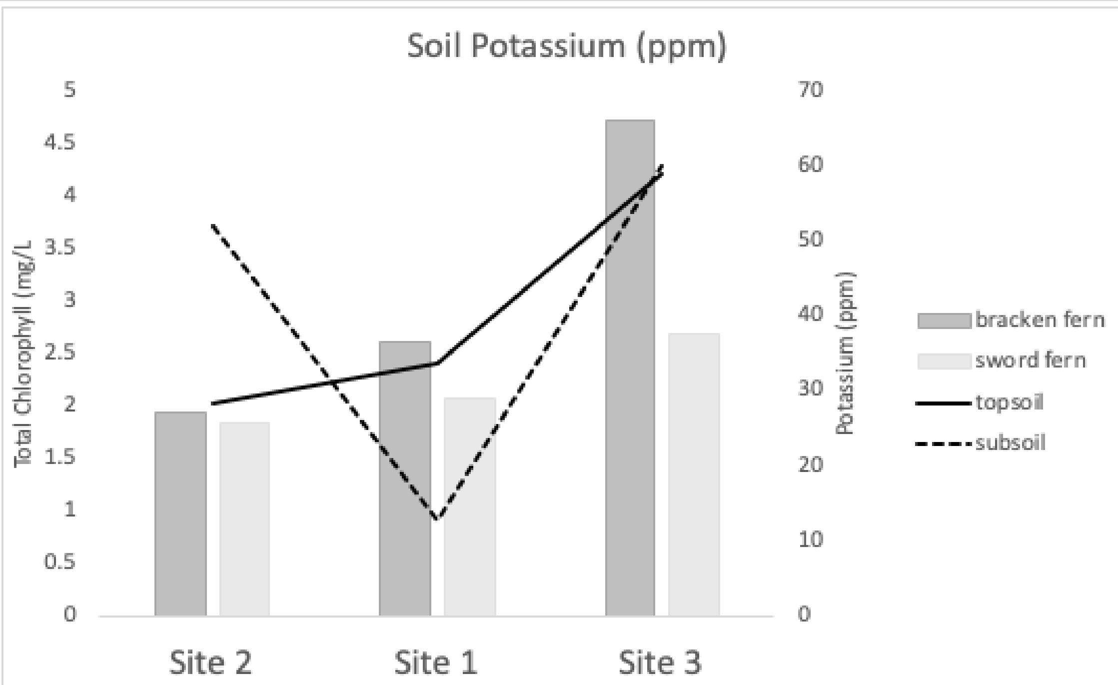
Soil Nutrient Analysis
Results of soil analysis are provided in Table 3. Lead was found in very low to no measurable quantity in both top and subsoil. Iron content at site 1 was the most abundant compared to the other sites followed by site 2 and site 3. Potassium was found to be most abundant in site 3 followed by site 1 and site 2. This trend was also found in calcium concentrations where site 3 was most abundant followed by site 1 and site 2.

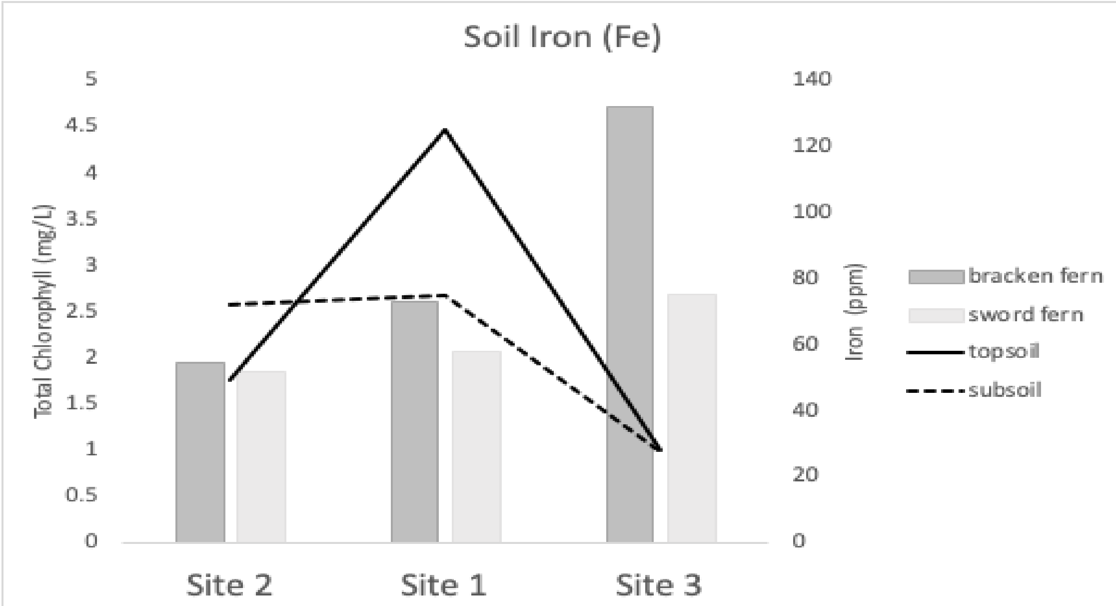
Table 3: AAS analysis of extractable minerals.

Sample	Lead (ppm)	Iron (ppm)	Potassium (ppm)	Calcium (ppm)
Site 1 Topsoil	0.18	125	33.6	1210
Site 2 Topsoil	0	49.1	28.2	215
Site 3 Topsoil	0.15	28.1	59.0	1575
Site 1 Subsoil	0	75.0	12.6	118
Site 2 Subsoil	0	72.0	52.0	1505

Site 3 Subsoil	0.17	27.5	60	1750







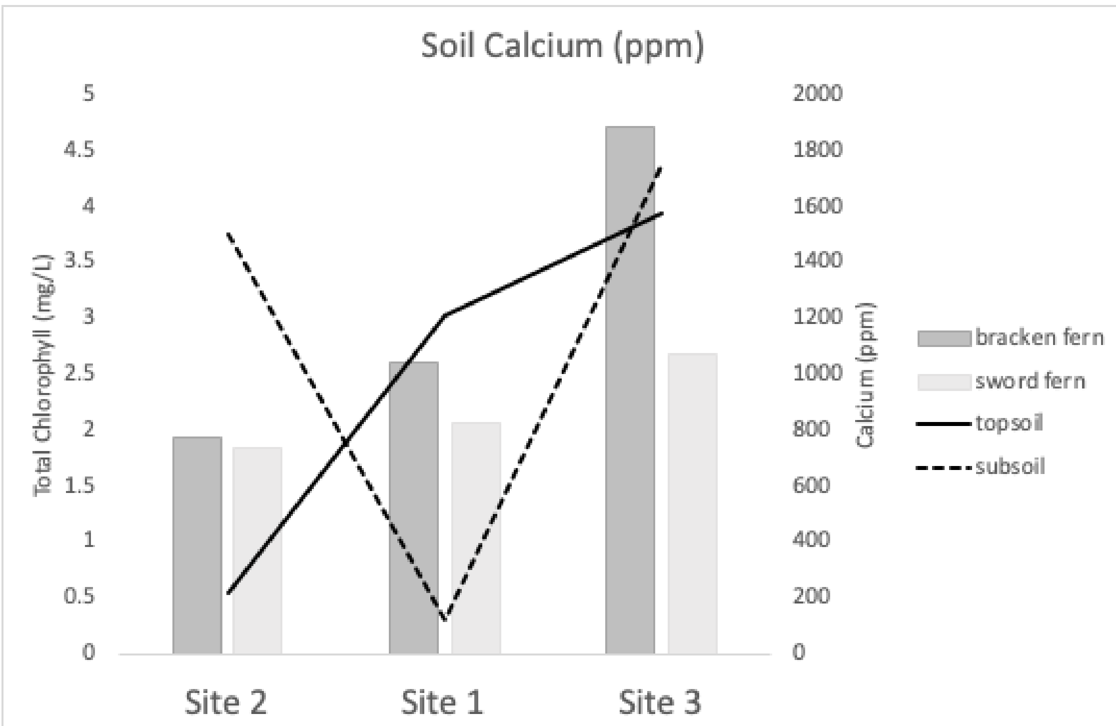


Figure 6. Comparison of nutrient concentrations to chlorophyll concentrations as mg/L of fern species individually **(a)** Nitrogen in frond tissue in percent, and soil concentrations in parts per million (ppm) for **(b)** potassium, **(c)** iron, and **(d)** calcium. The order of the sites has been rearranged in order of increasing chlorophyll levels to simplify comparisons. Vertical bars represent total chlorophyll concentration at each site for each species and correspond to the left axis. Lines represent specified nutrient content at each site and correspond to the left axis.

Discussion

There were some observable correlations between chlorophyll concentrations and the available minerals in the soils or fronds. The amount of nitrogen in the fronds, however, was not positively correlated with the calculated chlorophyll concentrations as expected (Fig. 6a). Even though nitrogen is present in the porphyrin ring of the chlorophyll structure, there is a higher amount of nitrogen in many other amino acids present in the fronds (Richardson 2010). The results of our analysis cannot clarify nitrogen in chlorophyll vs. other nitrogen present in the fronds. Extracting the chloroplasts from the fronds prior to nitrogen analysis would result in a more accurate comparison of nitrogen to chlorophyll concentration within the ferns themselves.
At each site location, there is a greater abundance of *Polystichum munitum* compared to *Pteridium aquilinum* and within the Arcata Community Forest overall. Interestingly, however, *Pteridium aquilinum* assimilated more nitrogen at each site. The better efficiency could result in greater adaptability to a wider variety of habitats, whereas *Polystichum munitum* is more limited to the moist, shady understory of coniferous and evergreen forests of western North America.

Potassium was found to be most abundant in site 3 followed by site 1 and site 2. Levels present in the topsoil had the strongest correlation with the total chlorophyll concentration of each element that was analyzed (Fig. 6b). Potassium is needed for the production of grana which is important for light dependent reactions during photosynthesis. A deficiency of potassium within the plant would result in a reduction of grana as well as little to no chlorophyll production. The levels of potassium in the soil are weakly correlated with the amount of nitrogen in both species that it is also needed for protein synthesis. The correlation with potassium in the soil and chlorophyll concentrations within the fronds cannot be directly compared since the amounts of potassium within the plants themselves were not recorded. If the levels of potassium in the soils were very low, we assumed that the ferns would be suffering from deficiency, but none of them were observed to be directly impacted.

As previously mentioned, iron content at site 1 was the most abundant compared to the other sites, but had no positive relationship with chlorophyll concentrations (Fig. 6c)). It is an important component of cytochromes and is an important electron transporter during photosynthetic electron transfers. Due to the requirement of iron for these processes, it was predicted that the iron levels in the soil would relate to chlorophyll concentrations, but this was not observed. Our study only looked at overall iron and not iron available for uptake by the plant. Future research should be conducted to identify the difference and how this affects the fern growth and development.

Calcium concentrations were most abundant at site 3 and the overall correlation was weak in the topsoil for both species, unlike what we hypothesized. The results from this study conducted are purely observational and are specific to the Arcata community forest. The actual causation for nutrient availability in the soils affecting fern development was not determined and should be investigated further.

Conclusion

After the series of analyses to answer our question on what affects soil nutrient levels have on chlorophyll production for our two species, it seems that there was little correlation. We hypothesized that nitrogen would show a positive correlation to chlorophyll concentrations, but our findings show no such association. Our soil analyses lead to us not finding any distinct correlation between calcium, potassium, or iron with chlorophyll production. This could immensely improve if given more time and changes to our experimentation. A larger quantity of samples from numerous plants would give us more representative data as well as a wider variety of sites within the Arcata community forest. A greater number of site locations and samples could result in a statistical significance between the different fern species that was not found in our study. It would have been preferable if we added our soil samples to the Kjeldahl organic nitrogen analysis alongside the plant matter in order to compare available nitrogen in the soil to nitrogen present in the ferns. Also, the plant matter could have been extended to our atomic absorption spectroscopy analysis, so we can cross reference our soil data with our plant matter data and deem if any of them were limited by available nutrients in the soil. For our nutrient analysis, Magnesium and Phosphorous should have been added to our study to check for a correlation with chlorophyll production. The study that we performed is a small step towards determining if available nutrients in the soil greatly affects the plants growth and the methods to survive in low nutrient environments.

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