A COMPARATIVE ANALYSIS OF FOUR METHODS FOR DETECTING NITROGEN IN A HETEROGENEOUS SERPENTINE LANDSCAPE: THE POTENTIAL INFLUENCE OF FIRE AND HABITAT

By

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ABSTRACT

A comparative analysis of four methods for detecting nitrogen in a heterogeneous serpentine landscape: the potential influence of fire and habitat

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Ultramafic parent materials in northern California and southwestern Oregon have given rise to one of the largest serpentine regions in the world. This area is dominated by sparse, fire-prone coniferous forests and shrublands which are apparently highly nutrient limited. Fire is the primary disturbance in these unique ecosystems, yet little is known about how fire influences soil nutrient dynamics. I utilized the large, ~202,000 ha Biscuit Fire of 2002 to compare nitrogen levels in burned versus unburned serpentine sites. A variety of methods are used to quantify nitrogen and nitrogen transformations in the field. Methods comparisons, however, are generally compared in a laboratory setting and rarely in the field. I compared three commonly used methods, plus a novel approach, to assess the effect of fire on two different habitats within a nutrient-poor serpentine landscape. I used soil sampling, ion exchange resin bags, and a hybrid soil sampling/resin bag method to compare burned and unburned areas five years after the fire. Nitrogen was quantified in both wetland and upland habitats, as these were expected to be affected differently by fire. No effect of the fire was detected in wetland habitats using any method, yet estimates of ambient levels of nitrogen varied among methods. There was, however, a mixed signal in the upland areas; the ion exchange resin
bags identified a significantly more nitrogen in unburned plots, while soil sampling detected no such difference. Additionally, no difference in soil nitrogen was detected between wetland and upland areas. My study demonstrates that conclusions about the role of fire on nitrogen dynamics can be strongly influenced by the methods of nitrogen detection used. Unlike comparisons carried out under controlled laboratory conditions, my work does not establish whether one method is better than another, only that it is possible to reach different conclusions depending which method one uses. Lastly, due to a lack of pre-burn data, as well as the five year time lag between the fire and sampling, and inconsistent estimates of soil nitrogen availability by various methods, my study does not provide firm conclusions about how fire affects nitrogen in serpentine habitats. To my knowledge, my study represents the first assessment of fire on soil nitrogen, as well as the first comparison between wetlands and uplands in serpentine habitats of northern California and Oregon. More research is needed to determine the effects of fire on soil nitrogen, and refinements need to be made to methodologies for quantifying nitrogen in these habitats. I discuss future research directions that could help elucidate the role of fire on nitrogen dynamics on serpentine soils as well as possible avenues for refining the methodologies for quantifying nitrogen.
DEDICATION

Dedicated to
Edward S. Franklin
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# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................. iii

DEDICATION ............................................................................................................................... v

ACKNOWLEDGEMENTS ........................................................................................................ vi

TABLE OF CONTENTS ........................................................................................................... vii

LIST OF TABLES ...................................................................................................................... ix

LIST OF FIGURES .................................................................................................................. x

INTRODUCTION ..................................................................................................................... 1

MATERIALS AND METHODS ............................................................................................... 10
  Study System ..................................................................................................................... 10
  Nitrogen Quantification .................................................................................................... 12
  Study Design ...................................................................................................................... 14
  Laboratory Procedures ..................................................................................................... 16
  Nitrogen Analysis .............................................................................................................. 17
  Statistical Analyses ............................................................................................................ 17
    N availability .................................................................................................................. 17
    N transformations .......................................................................................................... 19

RESULTS ................................................................................................................................... 23
  Between Habitat N Availability ....................................................................................... 23
  Within Habitat N Availability ......................................................................................... 23
  Single Method Analysis ................................................................................................. 24
  Nitrogen Transformations ............................................................................................... 25
DISCUSSION ................................................................................................................... 31

The Effect of Fire on Soil N............................................................................................ 31

Differences in Soil N Among Serpentine Habitats ...................................................... 35

Lysimeter Method ........................................................................................................ 37

Conclusions and Recommendations............................................................................ 39

LITERATURE CITED ..................................................................................................... 41
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Characteristics for serpentine sites where N was measured. A fen and upland plot were used (except where noted) at each site. Latitude and longitude are given as decimal degrees. Size refers to fen size only.</td>
</tr>
<tr>
<td>2</td>
<td>A three factor repeated measures ANCOVA run on both the soil core and the IER bag method simultaneously (** indicates significance at 0.05).</td>
</tr>
<tr>
<td>3</td>
<td>Results of two factor repeated measures ANCOVA for the lysimeter and ISC/IERB methods (** indicates significance at 0.05).</td>
</tr>
<tr>
<td>4</td>
<td>Univariate analyses for each nitrogen quantification method individually analyzing the difference in N between burned and unburned fens.</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>Devices utilized to quantify N. At left is a resin bag. They are filled with ~10 g each of anion and cation ion exchange resin, and installed directly in the soils of both fens and uplands. To the right of it is a soil core, which was used to sample soil in fens and uplands. At the upland sites, an additional soil core was installed, and capped with a resin bag at the bottom (the ISC/IERB method). At the right is a PVC housing that was installed in the fens as a container for an IER bag (resin based lysimeter). ........................ 21</td>
</tr>
<tr>
<td>2</td>
<td>Map of the study area used to assess N in serpentine fen and upland sites. Each symbol represents a fen-upland pair, where hollow symbols represent those sites that were burned in the 2002 Biscuit Fire, and solid symbols represent those that did not burn. ........................................................................ 22</td>
</tr>
<tr>
<td>3</td>
<td>Soil N availability as measured by soil cores and resin bags in fens (<em>Darlingtonia californica</em> symbol) and uplands (<em>Pinus jeffreyi</em> symbol). Upper panels represent soil N quantified by the soil core method, lower panels represent soil N as quantified by IER bag method.................................. 28</td>
</tr>
<tr>
<td>4</td>
<td>Soil N availability in fens and uplands varies by quantification method. Upper panels represent fen habitats, and lower panels represent upland habitats. Letters denote statistical significance at 0.05 for post-hoc multiple comparison tests for 2 factor ANOVA comparing fire and method. ............... 29</td>
</tr>
</tbody>
</table>
INTRODUCTION

The term ‘serpentine’ is used to describe one of the world’s most unique soil types, derived from ultramafic parent material generally made up of antigorite, chrysotile, and lizardite (Borine 1983, Mills and Roath 1984). Soils derived from serpentine bedrock tend to be high in heavy metals, characterized by a low nutrient content, and have a low Ca\(^{2+}\)/Mg\(^{2+}\) ratio (del Moral 1982, Turitzin 1982, Mansberg and Wentworth 1984). Vegetation on serpentine soils tends to be slow growing, of stunted appearance compared to vegetation on non-serpentine soils, and xeromorphic (Whittaker 1954, Proctor and Woodell 1975, Kruckeberg 1984). Macronutrient limitation (nitrogen and phosphorus) is generally considered to play a stronger role in controlling vegetation on these soils than the low Ca\(^{2+}\)/Mg\(^{2+}\) ratio or toxic elements in the soil (nickel, chromium, and cobalt; Proctor and Woodell 1975). Despite difficulties experienced by plants growing in these soils, serpentine regions almost universally harbor high levels of biological diversity and unique endemic species (Brooks 1987). For example, in California, plants endemic to serpentines make up 10% of the state’s flora, even though serpentine habitat occupies only 1.4% of the area in the state (Harrison et al. 2000, Wolf 2001).

Serpentine soils of northwestern California and southwestern Oregon harbor many unique vegetation types that are wholly or partially restricted to serpentine soils (Kruckeberg 1984). An example of one of these unique floristic components is
Darlingtonia californica (Sarraceniaceae), which grow in small, isolated, and perennially wet areas that are botanically distinct from the surrounding upland matrix where *D. californica* is absent (Whittaker 1960). The success of the insectivorous *D. californica* suggests that these ecosystems are limited by N and/or P, as carnivory is generally viewed as an adaptation to low soil nutrient levels that would be disadvantageous in more fertile soils (Ellison et al. 2003, Ellison and Farnsworth 2005). Fertilization experiments in nearby serpentine ecosystems support the hypothesis that these soils are N and/or P limited (Turitzin 1982, Huenneke et al. 1990). There have been no documented fertilization experiments in *D. californica* fens. *Darlingtonia californica* fens and the nearby uplands represent an opportunity to study two systems that are subject to the same macroenvironmental factors, yet are botanically distinct over distances of less than 10 m (Whittaker 1960, Kruckeberg 1984).

The primary disturbance in the serpentine ecosystems of California and Oregon is fire (Atzet and Martin 1991, Skinner 2003a, Skinner 2003b). Although little work has been done on fire frequencies on serpentine, some evidence indicates that fire differentially affects upland areas and fens (Skinner 2003a, Skinner 2003b, Cramer 2005). Before fire-suppression efforts, upland areas experienced a fire return interval eight to 30 years (Skinner 2003a) or eight to 15 years (Taylor and Skinner 2003), whereas fens experience fire every 18 to 42 years (Skinner 2003b). Given the large differences in soil moisture and organic content, it is likely that fen and upland soil N availability would be affected by fire in quite different ways. However, due to the dearth of studies in this region, little is known about the effect of fire on soil N.
Fire can induce a wide variety of chemical changes within ecosystems (Bond and van Wilgen 1996, Grogan et al. 2000). For instance, while fire can increase nutrient availability by releasing them from living vegetation into the soil (Raison 1979), it can also reduce aboveground vegetation, thus exposing the soil surface to increased erosion and nutrient losses (Debano and Conrad 1978, Raison 1979). Nitrogen is often a primary consideration when studying fire effects on soil nutrient composition as it often limits primary production (Wan et al. 2001). The effect of fire on N is complicated, as N can rapidly be lost to volatilization, nitrification, and leaching during combustion (Grier 1975, Debano and Conrad 1978, Vitousek and Howarth 1991), while at the same time rapid immobilization by microbes immediately following the fire may slow this loss of N (Seastedt and Hayes 1988). For instance, after a fire, nutrient rich ash that is deposited on the surface makes more N available to leaching, while also possibly acting as an immediate fertilizer for plants, expediting post burn vegetation recovery (Lewis 1974, Boerner 1982). Depending on the intensity of the fire, the soil substrate may be combusted, lowering the soil elevation (Wilbur and Christensen 1983). In some semiarid systems (not unlike the xeric landscape generally associated with serpentes), fire exerts a stronger control on N fluxes than does water, which is traditionally thought to control N fluxes in fen environments due to its role in leaching (Johnson et al. 1998).

Impacts of fire have been studied in a wide range of habitats (Whelan 1995), but due to variations in biotic characteristics (fuel load and/or presence of fire adapted species) and abiotic factors (topography, fire intensity, and climate), a wide range of outcomes and community responses have been described (Raison 1979, Bond and van
Thus, generalizations about the role of fire on soil nutrients are difficult to make, and it is crucial to continue to quantify the effects of fire in many diverse ecosystems (Grogan et al. 2000). Studies on the fire effects on serpentine plant communities are rare, where the effects of fire on serpentine plant succession and diversity have been described (McCoy et al. 1999, Safford and Harrison 2004), but fire effects on soil N dynamics have not been addressed.

Nitrogen plays a key role in many biological processes and is a limiting nutrient in many terrestrial ecosystems (Chapin III et al. 1986, Vitousek and Howarth 1991, Vitousek and Field 1999), including most serpentine ecosystems (Proctor and Woodell 1975, Turitzin 1982, Huenneke et al. 1990). Due to the importance of N for ecosystem productivity, understanding soil N has been an integral part of ecological science since quantification methods were developed (Keeney 1980, Binkley and Hart 1989). Over the past 50 years, there has been a proliferation of methods used to quantify soil N availability and N transformations, such as mineralization, both in situ as well as in laboratory settings. Methods used to assess soil N include, for example, tension lysimeters, simple soil collection, and the application of synthetic resins (Keeney 1980, Binkley and Hart 1989). Understandably, most studies of terrestrial ecosystems choose only one method, which provides few opportunities to compare methods within a single study. Comparisons of methods are generally restricted to summary articles, meta-analyses, and laboratory studies; where in situ comparative experiments are rare (Keeney 1980, Binkley and Hart 1989). There are a few studies, however, that compare methods directly to one another (for examples see Binkley and Matson 1983, Raison et al. 1987,
Hart and Firestone 1989, Knoepp and Swank 1995, and Brye and Slaton 2003, Johnson et al. 2005), but results aren’t consistent across studies and further explorations and comparisons are necessary, especially to expand the range of ecosystems explored. Additionally, these studies are generally biased towards N-saturated agricultural systems (Brye and Slaton 2003), or ecosystems where N is not considered to be a limiting factor (Binkley et al. 1986, Raison et al. 1987, Adams et al. 1989, Binkley et al. 1992, Knoepp and Swank 1995, Johnson et al. 2005).

While there are many ways to quantify N, the most direct method is by collecting soil directly, extracting it with a strong salt either in the field or in the lab to mimic plant uptake (Bremner 1965), and using an autoanalyzer to quantify N levels in the solution. Soil cores are generally collected using a metal or plastic polyvinyl chloride (PVC) tube, though PVC has become the preferred medium due to its low cost, chemical inertness (galvanized metal is Zn coated which may inhibit soil N mineralization), and thermal properties (Raison et al. 1987, Binkley and Hart 1989). A less labor intensive method involves the use of synthetic ion exchange resins (IER). Ion exchange resins are synthetically manufactured substances that have a highly adsorptive surface, large surface area, and a strong negative or positive surface charge. Ion exchange resins placed in bags in the soil adsorb ions from solution, which can be extracted later with a strong salt solution (Binkley and Hart 1989). The latter is then assayed for ion content using an autoanalyzer. Ion exchange resins have found a practical application in ecological research for over 50 years (Amer et al. 1955, Sibbeson 1977, Qian and Schoenau 2002), and provide a way to measure nutrient availability with minimal cost, labor, and soil
disturbance (Binkley and Hart 1989). Other methods, such as collecting
groundwater/porewater using lysimeters or soil sampling with soil cores are hampered by
short time intervals between successive collections, whereas resins can be left in the field
for up to a year (Susfalk and Johnson 2002). Additionally, ion exchange resins are more
sensitive to on site factors than other methods simply because they are left to absorb N
over a time period when site factors, like precipitation, may change. Furthermore, IER
bags may be more sensitive than soil sampling to differences in net N mineralization, ion
mobility, moisture regime, and competition for N (Binkley and Matson 1983, Hart and
Firestone 1988).

In addition to N availability, many researchers are interested in N transformations,
such as N mineralization and nitrification, and again, there are a variety of methods
available for both in situ and laboratory incubations. Laboratory incubations (either
aerobic or anaerobic), while good for gaining an idea of soil N transformation potential
under optimal conditions, tend to lack the relevance of in situ incubations where soils are
exposed to natural temperature fluctuations as well as (in some methods) natural moisture
regimes (Hart and Firestone 1989). Two of the most popular and widely applied methods
used to quantify N transformations are the buried bag method (i.e. placing soil in
polyethylene bags and incubating them in situ, Eno 1960) and isotopic pool dilutions
using $^{15}$N methods. The latter involve injecting labeled isotopes of N that naturally occur
at very low concentrations into the soil and tracking the ratio of $^{15}$N:native $^{14}$N as the $^{14}$N
is metabolized by microbes into other forms, thus increasing the relative percentage of
$^{15}$N in the soil (Davidson et al. 1991). These two methods are widely considered to be
the most accurate for measuring gross N transformations over time. Both methods have
their drawbacks, as buried polyethylene bags isolate the soil from any moisture
variations, which may strongly influence N dynamics (Binkley and Hart 1989). Isotopic
\(^{15}\)N methods can be expensive and require quantification of background levels in
various N pools, which can be difficult and time consuming (Högberg 1997). However,
there is a hybrid method of measuring N transformations, known as the in-situ-soil-
core/ion-exchange-resin-bag (ISC/IERB) method, which was developed by Di Stefano
and Gholz (1986) and refined by Brye et al. (2002b). After using a PVC tube to collect a
soil core, the intact PVC tube/soil core assembly is ‘capped’ at the bottom with a resin
bag and replaced into the hole in the ground the original soil core created, similar to the
buried bag method (Eno 1960). This setup allows the resin bag to trap any nutrients
leaching out of the soil core and allows the researcher to quantify nutrient
transformations within the PVC core, while maintaining ambient soil temperature and
moisture regimes. Additionally, since the IER bag is situated entirely within the PVC
core, it is protected from root penetration and local weather conditions that would cause
the bag to disintegrate (Susfalk and Johnson 2002). Net N mineralization is calculated as
the sum of ammonium nitrogen \((\text{NH}_4^+\text{-N})\) and nitrate nitrogen \((\text{NO}_3^-\text{-N})\) over time:

\[
\text{Net N mineralization} = \left(\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}\right)_{\text{Final}} - \left(\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}\right)_{\text{Initial}} + \left(\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}\right)_{\text{Resin}}
\]

after Brye et al. (2003). Net nitrification is calculated the same way, omitting the \(\text{NH}_4^+\text{-N}\) in each term.
A major challenge inherent in quantifying soil N by any method is that nutrients are not evenly distributed throughout the soil profile (Binkley and Hart 1989). Additionally, if a sampling regime is maintained for a long enough period of time, the repeated samplings may disturb the substrate, and hence alter N cycling regimes and soil N levels. I sought to design a method for deploying IER bags that would simultaneously control for soil heterogeneity as well as minimize soil disturbance attributed to sampling. I developed a method to use a PVC housing to act as a semi-permanent site installation for an IER bag in areas with significant groundwater flows. This new method, termed a resin-based lysimeter, can be repeatedly sampled without disturbing the soil profile, except for the initial installation.

Ion exchange resins have been applied in a lysimeter format (a device for collecting soil water, generally with the intent of testing the collected water for nutrient concentrations) to sample soil solution twice before (Sakadevan et al. 1994, Susfalk and Johnson 2002). One study used a highly invasive (significant perturbation of the soil profile) lysimeter setup (Sakadevan et al. 1994), whereas the other was used to quantify solution N in snowmelt (Susfalk and Johnson 2002). This is the first time a resin-based lysimeter (hereafter lysimeter) has been implemented to sample soil solution N in wetlands.

I took advantage of a large natural fire that burned on serpentine soils in California and Oregon in 2002 to assess the effect, if any, the fire had on soil N availability and transformations in both xeric upland and mesic fen habitats. Despite sampling five years after the fire (2007), I had reason to believe that any differences
between burned and unburned sites would still be evident, as N cycling can take longer than four years to recover following some fires (Christensen and Muller 1975, Binkley et al. 1992, Monleon et al. 1997, Brye et al. 2002a, Knoepp et al. 2004, Sherman et al. 2005). My work addressed two primary questions. First, does N availability in serpentine habitats vary between habitat types (fen vs. upland) and burned and unburned sites? Second, how do different methods (Figure 1) of quantifying N influence the detection of variation in N? In other words, would different conclusions be made about the effect of fire and habitat on N availability if only one method had been used?
MATERIALS AND METHODS

Study System

My study was conducted in the Siskiyou Mountains of northern California and southwestern Oregon during the summer of 2007 (Figure 2). The climate of this area is Mediterranean, with cool winters (mean January minimum temperature = 0° C) and warm dry summers (mean July maximum temperature = 31.7° C; mean annual precipitation = 154 cm, with only 4 cm falling between June and August). Soils in southwestern Oregon are in the Pearsoll Series (clayey-skeletal, magnesic, mesic Lithic Dystroxerepts), Eightlar Series (clayey-skeletal, magnesic, mesic Typic Dystroxerepts), or Dubakella Series (clayey-skeletal, magnesic, mesic Mollic Haploxeralfs; Borine 1983) whereas soils in northern California are in the Walnett Series (loamy-skeletal, parasesquic, mesic Ultic Haploxeralfs) or Jayell Series (fine, parasesquic, mesic Typic Dystroxerepts; Mills and Roath 1984). All soils in the study area are rocky, well drained, and no deeper than 155 cm (but generally closer to 81 cm deep) (Borine 1983, Mills and Roath 1984). Upland habitats within the study area are characterized by a sparse tree layer of *Pinus jeffreyi*, *Pinus attenuata*, and to a lesser extent *Pseudotsuga menziesii*. A well-developed shrub layer is dominated by *Arctostaphylos spp.*, *Quercus vaccinifolia*, *Lithocarpus densiflorus var. echinoides*, and *Rhamnus californica*. There is also a diverse layer of ephemeral spring forbs and graminoids that senesce by mid-summer (Whittaker 1960). While the term ‘fen’ may not be accurate as a technical term for these wetlands (not all of them exhibited the significant organic soil development that are a trademark of fens), the term
has been used historically to describe wetland areas in northern California and southwestern Oregon where *D. californica* grows (Whittaker 1960), and I will use this convention from hereon. The fens are characterized by a highly diverse assemblage of forbs and graminoids in addition to *D. californica* that are distinct from the upland habitats (Whittaker 1954, Whittaker 1960, Frost and Jules 2007) and a water table that is at or within a few centimeters of the surface (D. Franklin *pers. obs*.). Overstory vegetation in the fens is sparse and generally limited to marginal areas, however there are occasional *Chamaecyparis lawsoniana* growing directly in the fens (Cramer 2005, Frost and Jules 2007).

A large forest fire burned approximately 202,000 ha of the Siskiyou Mountains of northern California and southwestern Oregon (the Biscuit Fire; USDA Forest Service and BLM 2004) from July to September 2002. Much of this area is underlain by serpentine parent material. The distribution of serpentine outcrops, due to generally shallow bedrock, had a profound effect on the fire, resulting in a very heterogeneous pattern of burning, where some areas burned much more intensely than others (Thompson et al. 2007).

Nitrogen Quantification

Soil N levels were quantified four different ways. Ion exchange resin bags were constructed out of nylon stockings and fishing line with 10 grams each of REXYN™ Amberlite IRA 402 Anion Resin and REXYN™ Amberlite IRA 200 Cation Resin (Alfa Aesar, Ward Hill, MA, USA; Figure 1). Any ions already on resins were removed by
soaking them in 2N KCl for at least 24 hours prior to deployment, then rinsing with distilled deionized water. These were buried in the soil to a depth of 10-15 cm, depending on substrate availability. Additionally, soil samples were collected using PVC cores. These cores are 5.2 cm by 30 cm, beveled at one end to minimize soil compaction on installation (after Brye and Slaton 2003, Figure 1), and they were driven into the soil with a rubber mallet to a depth of 22 cm. The PVC core and soil were then removed, and the soil inside the core was collected into sterile Whirl-Pak™ bags (Nasco, Fort Atkinson, WI, USA).

The third method for quantifying soil N is the ISC/IERB method (see Introduction), which was used to gain an understanding of N transformations. Soils were collected following the same method as the soil cores, however, a soil core capped with a resin bag was left to incubate at the sites between sampling dates. Finally, PVC housings (resin based lysimeters; Figure 1) were buried in the soil to act as permanent housings for IER bags. These were 10 cm by 35.5 cm with 5-7 cm slits in the side and bottom to allow water flow over an IER bag placed in the bottom of the chamber and were buried to a depth of 42 cm, unless the soil substrate was too shallow or rocky, in which case they were buried to a depth of 35 cm. Side slits were never exposed above the soil surface at either depth. The housings were capped with a PVC screw top to prevent atmospheric deposition of N as well as any other possible contamination. In summary, the four methods used were buried IER bags, PVC soil core, the ISC/IERB method, and resin based lysimeters (PVC housings with an IER bag inside (Figure 1)).
Study Design

Five *D. californica* fens were selected within the area burned during the Biscuit Fire, and five fens were selected in adjacent unburned areas (Figure 2). The fens occur across a variety of elevations and across a broad geographical area (see Ratchford et al. 2005, Sanders et al. 2007). Study areas were relatively undisturbed by human activity and were large enough to contain an 8 × 8 m sampling plot for earlier studies (Ratchford et al. 2005, Sanders et al. 2007). Every effort was made to quantify N as close to these pre-existing plots as possible. In addition, at both burned and unburned sites, an upland plot was selected, located at least 50 m away from the boundary of one of the study fens in a direction that placed the plot at approximately the same elevation, slope, and aspect as the fen plot. If more than one direction from the fen fit these criteria, then the direction was randomly chosen for the placement of the upland plot.

Three of the four methods were used to assess N in each fen or upland site. In the fens, I used the buried IER bag method, sampled soil using a PVC core, and the resin based lysimeter. In the uplands, I used the buried IER bag method, sampled soil using a PVC core, and the ISC/IERB method. Due to lack of groundwater flow in the uplands, the lysimeter method was only used in the fen areas. Additionally, the ISC/IERB method was only used in the upland areas due to the difficulties in retrieving the bottom resin from the wet, clayey substrate of the fens, as well as concerns about the high likelihood of vertical transport of soil water into the bottom of the soil cores.

Each suite of three methods (referred to as one installation) was replicated four times in each fen and upland plot, in a row perpendicular to the slope, and spaced at least
6 m from each neighbor in order to encompass as much site variability as possible as well as to prevent sampling the same groundwater as it percolates downslope. In one case, the fen was smaller than the required 18 m, so installations were spaced approximately 4 m apart. Following initial installation, sites were visited three times during the summer of 2007, every four weeks. Because of the difficulty in accessing remote sites, and in processing large volumes of samples, the number of sites studied was small (n=10 for fens and n=10 for uplands, where five of each were burned), and I have taken a conservative approach to interpreting my data.

Researchers wore latex gloves whenever handling samples in both the field and the laboratory to prevent contamination. Since there were no easily discernable soil horizons present, all soils inside the core were collected and homogenized. All collected soils and resins were kept cold (~5°C) until they could be processed, generally 72 hours at most.

From August to September 2006, installations were placed in seven (out of an eventual total of ten) fen-upland pairs. Four fens-upland pairs were in California in the Smith River National Recreation Area, and three were in the vicinity of Cave Junction, Oregon (Figure 2. Due to time constraints, the remaining three fen-upland pairs were installed in late April-early May 2007 in conjunction with the commencement of sampling, and were located in the Cave Junction area.
Laboratory Procedures

In the laboratory, all containers and filters were acid washed with 2 N HCl and rinsed three times with DI in order to remove any trace nutrients that may have contaminated samples. All soils from cores and the ISC/IERB methods were sieved to 5 mm to remove large rocks, plant roots, and any other biological debris as well as to homogenize the sample. A subsample of approximately 20 g was removed from the total sample for measurement of gravimetric water content. All soils were dried in aluminum tins for 36 hours at 60-75 °C, and then reweighed after cooling. A second subsample of approximate 20 g of field wet soil was added to 100 ml of 2 N KCl, shaken vigorously, then statically incubated for 48 hours. Samples were then filtered by vacuum filtration (BD Falcon Filters, Franklin Lakes, NJ, USA with Whatman GF/F 7 µm glass fiber filters, Fisher Scientific, Pittsburgh, PA, USA), and the filtrate collected for analysis.

All collected IER bags that were either buried directly in the soil, from inside the lysimeters, or from the bottom of the ISC/IERB core were dried in aluminum tins at 60-75 °C for 36 hours, weighed, added to 100 ml of 2 N KCl, shaken vigorously, and statically incubated for 48 hours. The IER bags were then removed from solution, and the solute collected and stored for future analysis. Some IER bags broke, either over the course of incubation due to a variety of site factors (i.e. shearing on a rock during installation or puncturing by an actively growing plant root), or were damaged in the process of collecting them. When this occurred, every attempt was made in both the field and laboratory to retrieve as much resin as possible from broken IER bags. In the case of
broken bags, the resin-KCl mixture was vacuum filtered according to the same protocol as the soils, and the resins discarded. Intact IER bags were thoroughly rinsed in distilled deionized water, then re-immersed in 2N KCl for at least 24 hours prior to redeployment in the field.

Nitrogen Analysis

All samples were analyzed on a segmented flow autoanalyzer (Bran+Luebbe AA3, Norderstedt, Germany) for NH$_4^+$ (berthelot reaction, method G-175-96 Rev 12), NO$_3^-$+NO$_2^-$ (cadmium reduction followed by azo dye formation, method G-172-96 Rev 10), and NO$_2^-$ (azo dye formation, method G-173-96 Rev 8). NO$_3^-$ and NO$_2^-$ peak absorbance was measured at 550 nm, and NH$_4^+$ peak absorbance was measured at 660 nm. Samples were run with a 2N KCl buffer to match the filtrate concentrations. NO$_3^-$ content was determined by subtracting NO$_2^-$ from NO$_3^-$+NO$_2^-$ measurements.

Statistical Analyses

N availability

Throughout my analyses, all tests will be run for NH$_4^+$-N and NO$_3^-$-N separately, as these represent two different pools of plant available N present in the soil. Thus, each individual statistical test was run twice, once for NH$_4^+$-N and once for NO$_3^-$-N. Since samples were collected through the growing season, I first used repeated measures ANCOVA to assess what role sample collection date had on soil N availability. Because the soil core method and the buried IER bag method were replicated in each habitat, I
assessed N measures derived from these methods simultaneously. In these ANCOVAs, the main factors were habitat type (fen vs. upland), fire treatment (burned vs. unburned) and method (buried IER bag vs. soil core). Covariates included were elevation and distance to the coast (Table 1). Both elevation and distance to the coast are suspected to play a significant role in governing fire intensity, and distance to coast is known to have a strong influence on vegetation (Frost et al. 2004). Additionally, fens were not evenly distributed along a longitudinal gradient (mean distance to coast_{burned} = 29.7 km, mean distance to coast_{unburned} = 36.8 km, Figure 2). I also ran two-factor repeated measures ANCOVAs for the other two methods (lysimeter and ISC/IERB) separately, omitting habitat as a main factor. Each method was analyzed twice, once for NH$_4^+$-N, and once for NO$_3^-$-N. Covariates remained the same in these later tests.

Because date of sampling did not influence either NH$_4^+$-N or NO$_3^-$-N, the data were averaged over the growing season to produce one measure per site for each method. Next, for the soil core method and buried IER bag method, a three factor ANCOVA was used to assess the effect habitat, burn history, and method had on NH$_4^+$-N and NO$_3^-$-N availability. I then used a two factor ANCOVA to assess the effect of sample method and fire on NH$_4^+$-N and NO$_3^-$-N in each habitat separately. Covariates used in the ANCOVAs were the same as in the repeated measures analysis, however, the size of the fen was included as a covariate for fen analyses. Pairwise comparisons were made with Fishers least significant difference multiple comparison post-hoc test.

In order to determine how using a single method of N quantification would affect interpretation of fire effects in this ecosystem, a series of one factor ANOVAs were used
to assess differences in both $\text{NH}_4^+$-N and $\text{NO}_3^-$-N in burned and unburned plots. One test was run for each method in each habitat.

**N transformations**

I used a one way ANCOVA to assess the effect of fire on N mineralization and nitrification. Elevation, distance to coast, and soil moisture were used as covariates. Soil moisture was included as a covariate due to its strong role in controlling N transformations (Binkley and Hart 1989). All statistical tests were conducted using Number Cruncher Statistical Systems 2004 (Hintz 2004), except for the repeated measures ANCOVA, which was conducted using SPSS (SPSS 2008). All data met the parametric assumptions for ANOVA tests. Additionally, results for interaction terms and covariates that were not significant at at least 0.1 are not reported.
Table 1: Characteristics for serpentine sites where N was measured. A fen and upland plot were used (except where noted) at each site. Latitude and longitude are given as decimal degrees. Size refers to fen size only.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Burn Status</th>
<th>Latitude (dd)</th>
<th>Longitude (dd)</th>
<th>Elevation (m)</th>
<th>Distance to coast (km)</th>
<th>Size (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Root</td>
<td>Burned</td>
<td>41.979</td>
<td>-123.951</td>
<td>434.8</td>
<td>21.092</td>
<td>250</td>
</tr>
<tr>
<td>Dragonfly</td>
<td>Unburned</td>
<td>41.896</td>
<td>-124.002</td>
<td>512.5</td>
<td>16.979</td>
<td>950</td>
</tr>
<tr>
<td>Greg's</td>
<td>Burned</td>
<td>41.977</td>
<td>-123.949</td>
<td>524.9</td>
<td>21.293</td>
<td>500</td>
</tr>
<tr>
<td>L.E. Horton</td>
<td>Burned</td>
<td>41.937</td>
<td>-124.005</td>
<td>489.7</td>
<td>16.523</td>
<td>800</td>
</tr>
<tr>
<td>Lone Mountain</td>
<td>Unburned</td>
<td>42.051</td>
<td>-123.745</td>
<td>494.7</td>
<td>45.411</td>
<td>1500</td>
</tr>
<tr>
<td>Maureen’s (Wetland only)</td>
<td>Unburned</td>
<td>42.240</td>
<td>-123.675</td>
<td>409.5</td>
<td>36.109</td>
<td>200</td>
</tr>
<tr>
<td>New (Upland only)</td>
<td>Unburned</td>
<td>42.023</td>
<td>-123.773</td>
<td>550.5</td>
<td>23.258</td>
<td>200</td>
</tr>
<tr>
<td>South Side</td>
<td>Unburned</td>
<td>42.233</td>
<td>-123.659</td>
<td>423.5</td>
<td>60.550</td>
<td>1500</td>
</tr>
<tr>
<td>Upper Days Gulch 1</td>
<td>Burned</td>
<td>42.229</td>
<td>-123.713</td>
<td>474.6</td>
<td>55.478</td>
<td>900</td>
</tr>
<tr>
<td>Upper Days Gulch 2</td>
<td>Burned</td>
<td>42.227</td>
<td>-123.713</td>
<td>473.2</td>
<td>34.131</td>
<td>900</td>
</tr>
<tr>
<td>Whiskey Creek</td>
<td>Unburned</td>
<td>42.023</td>
<td>-123.773</td>
<td>524.6</td>
<td>38.573</td>
<td>875</td>
</tr>
</tbody>
</table>
Figure 1: Devices utilized to quantify N. At left is a resin bag. They are filled with ~10 g each of anion and cation ion exchange resin, and installed directly in the soils of both fens and uplands. To the right of it is a soil core, which was used to sample soil in fens and uplands. At the upland sites, an additional soil core was installed, and capped with a resin bag at the bottom (the ISC/IERB method). At the right is a PVC housing that was installed in the fens as a container for an IER bag (resin based lysimeter).
Figure 2: Map of the study area used to assess N in serpentine fen and upland sites. Each symbol represents a fen-upland pair, where hollow symbols represent those sites that were burned in the 2002 Biscuit Fire, and solid symbols represent those that did not burn.
RESULTS

Between Habitat N Availability

There was no effect of sample date on NH$_4^+$-N or NO$_3^-$-N, as detected by any method (Tables 2 and 3), thus all subsequent results are for values for each site, averaged across sampling date for each form of N. After averaging across sample date, habitat type also had no effect on soil N availability (NH$_4^+$-N: $F_{1,40}=0.004$, $p=0.952$; NO$_3^-$-N: $F_{1,40}=0.07$, $p=0.800$; Figure 3). However, the buried IER bags detected significantly more NO$_3^-$-N than the soil core method ($F_{1,40}=5.75$, $p=0.023$) across both habitats; buried IER bags detected 62% more NO$_3^-$-N than soil cores.

Within Habitat N Availability

In order to include methods that were not replicated in each habitat, the following analyses deal with fen and upland habitats separately. In the fens, there was no significant difference in NH$_4^+$-N between burned and unburned sites ($F_{1,29}=0.26$, $p=0.613$) or between methods of measuring N ($F_{2,29}=0.61$, $p=0.553$; Figure 4). The buried IER bag detected 47% more NO$_3^-$-N than the soil core, however neither method differed significantly from the lysimeter method ($F_{2,30}=4.41$, $p=0.025$, Fisher’s LSD significant at 0.05, Figure 4). There was no detectable difference in NO$_3^-$-N between burned and unburned sites ($F_{1,30}=0.08$, $p=0.779$). One of the covariates, the size of the fen, had a significant relationship with NO$_3^-$-N ($F_{1,30}=5.53$, $p=0.029$).
In the uplands, there was significantly less NH$_4^+$-N in the burned sites (F$_{1,30}$=5.93, p=0.023, Figure 4), where burned sites had 70% less NH$_4^+$-N, but the three methods did not vary in the amounts of NH$_4^+$-N they detected (F$_{2,30}$=0.04, p=0.965). There was no significant difference between burned or unburned sites, or between any of the three methods in terms of NO$_3^-$-N in the upland areas (Fire F$_{1,30}$=1.18, p=0.289; Method F$_{2,30}$=0.63, p=0.543, Figure 4). A marginally significant interaction between fire and method was detected for NO$_3^-$-N (F$_{2,30}$=2.58, p=0.098). Additionally, one covariate, distance to the coast, had a strong relationship with NO$_3^-$-N (F$_{1,30}$=9.10, p=0.006) in the upland areas.

**Single Method Analysis**

The difference in NH$_4^+$-N detected between burned and unburned plots by the buried IER bag method was the main reason that I found a significant difference between burned and unburned plots in the upland areas, as well as the significant interaction terms for upland NO$_3^-$-N. In the upland plots, the buried IER bag method detected significantly more NH$_4^+$-N (F$_{1,10}$=6.27, p=0.0367, Table 4) and NO$_3^-$-N (F$_{1,10}$=8.6, p=0.019, Table 4) in the unburned areas. There was 54% less NH$_4^+$-N and 81% less NO$_3^-$-N in the burned areas. No other single method detected any difference between burned and unburned plots (Table 4).
Nitrogen Transformations

There was no difference between burned and unburned plots in terms of N mineralization ($F_{1,10}=0.49$, $p=0.515$) or nitrification ($F_{1,10}=2.43$, $p=0.180$). The overall rate of net N mineralization was very low in both habitats; there was 0.003 mg g$^{-1}$ month$^{-1}$ mineralized in the burned areas, and -0.005 mg g$^{-1}$ month$^{-1}$ in the unburned areas (the negative value representing N sequestration). However, both of these values are less than the standard error (0.007). There was 0.003 mg g$^{-1}$ month$^{-1}$ of nitrification in the burned areas, and 0.001 mg g$^{-1}$ month$^{-1}$ in the unburned areas (standard error = 0.001).
Table 2: A three factor repeated measures ANCOVA run on both the soil core and the IER bag method simultaneously (** indicates significance at 0.05).

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
<th>P</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
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<td>0.030**</td>
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<tr>
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</tr>
<tr>
<td><strong>Nitrate (NO₃⁻-N)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>3.59 x 10⁻⁵</td>
<td>0.178</td>
<td>0.838</td>
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<tr>
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</tr>
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<td>7.165</td>
<td>0.013**</td>
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<td>Error</td>
<td>26</td>
<td>5.73 x 10⁻³</td>
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</table>
Table 3: Results of two factor repeated measures ANCOVA for the lysimeter and ISC/IERB methods (** indicates significance at 0.05).

<table>
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<tr>
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<th>P</th>
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<td><strong>Lysimeter Ammonium (NH₄⁺-N)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Time</td>
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<td>7.22 x 10⁻⁵</td>
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<tr>
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<td>1</td>
<td>2.29 x 10⁻⁴</td>
<td>0.429</td>
<td>0.548</td>
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<tr>
<td>Error</td>
<td>4</td>
<td>2.14 x 10⁻³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lysimeter Nitrate (NO₃⁻-N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>7.20 x 10⁻⁵</td>
<td>0.218</td>
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</tr>
<tr>
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<td>4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>ISC/IERB Ammonium (NH₄⁺-N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Elevation</td>
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<tr>
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<tr>
<td><strong>ISC/IERB Nitrate (NO₃⁻-N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
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<td>3.41 x 10⁻⁴</td>
<td>2.311</td>
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<tr>
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<td>1.51 x 10⁻⁴</td>
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<tr>
<td>Error</td>
<td>5</td>
<td>2.56 x 10⁻⁴</td>
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Figure 3: Soil N availability as measured by soil cores and resin bags in fens (*Darlingtonia californica* symbol) and uplands (*Pinus jeffreyi* symbol). Upper panels represent soil N quantified by the soil core method, lower panels represent soil N as quantified by IER bag method.
Figure 4: Soil N availability in fens and uplands varies by quantification method. Upper panels represent fen habitats, and lower panels represent upland habitats. Letters denote statistical significance at 0.05 for post-hoc multiple comparison tests for 2 factor ANOVA comparing fire and method.
Table 4: Univariate analyses for each nitrogen quantification method individually analyzing the difference in N between burned and unburned fens.

<table>
<thead>
<tr>
<th>Source</th>
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<tr>
<td>Fen Ammonium (NH₄⁺-N)</td>
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<td></td>
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<tr>
<td>IER Bag</td>
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<td>IER Bag</td>
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<td>8.600</td>
<td>0.019**</td>
</tr>
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<td>Upland Nitrate (NO₃⁻-N)</td>
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</tr>
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<td>IER Bag</td>
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<tr>
<td>ISC/IERB</td>
<td>1</td>
<td>3.06 x 10⁻⁶</td>
<td>0.080</td>
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DISCUSSION

The Effect of Fire on Soil N

Did the large Biscuit Fire event of 2002 alter N dynamics in serpentine habitats of northern California and southern Oregon? Perhaps the most important result of my work is that the answer to this question depends on the method of measuring N. My analyses detected an effect of fire in the upland sites and not in the fens. However, this effect was limited to the buried IER bag method. In fact, after removing this method from my analyses, there was no detectable effect of fire in either habitat of any form of N I measured, by any quantification method. One caveat is that two of the different methods, buried IER bag and soil sampling, measure slightly different pools of available soil N. The soil core acts as a ‘snapshot’ of what is available at the moment it is collected, where the buried IER bag gives a slightly longer term picture over the course of the incubation and may overrepresent labile N pools (Binkley and Hart 1989). Nevertheless, any difference between burned and unburned sites should have been detected by both methods, as generally when these two methods are deployed in concert, they report similar results (e.g. Hart and Firestone 1989, Johnson et al. 2005, Turner et al. 2007).

An alternate explanation for the difference between burned and unburned sites detected by the IER bags is that they may have been picking up on other, more subtle, non-fire related N fluctuations. Ion exchange resin bags are thought to be more sensitive to differences in on-site factors (Binkley and Matson 1983), and may have been influenced by some other site characteristic, such as a longitudinal gradient, rather than
any legacy effect of the Biscuit Fire. IER bags adsorb more ions from solution as soil moisture increases (Wyland and Jackson 1993). As one moves away from the coast, elevation and rainfall generally decrease. The majority of the unburned sites are located east of the burned sites, so the burned sites may have experienced higher leaching during the spring rains, lowering soil N prior to sampling. Thus, when I sampled during summer, I may have been detecting labile N still present in unburned sites that would have been leached out of the more coastal burned sites earlier in the season prior to sampling.

While it is difficult to make firm conclusions about whether there are still measurable differences in soil N between burned and unburned sites five years after the Biscuit Fire, one thing is clear: if I had just sampled soils (as is common in many studies; see Richter et al. 1982, Matson et al. 1987, Tilman and Wedin 1991, Morghan and Seastedt 1999, Suding et al. 2004 for examples), I would likely have concluded that there was no detectable difference between burned and unburned sites. Additionally, I would have concluded that burned sites had returned to N dynamics similar to the unburned sites in the five years since the fire. Studies that utilize solely the IER method are much less common (see Miller et al. 2006), but if I had used only IER bags, I would have come to a very different conclusion. I would speculate that N dynamics in the fens had either completely recovered or were only marginally affected by the fire, and that the uplands had likely not recovered. Hypothesizing that there might be a detectable difference in soil N availability between burned and unburned sites, this conclusion would have fit nicely with common sense about the fens. Due to a lack of combustible materials and the
presence of cool, running water, the fens may not have experienced any appreciable loss or release of N due to fire. Because the fire did not alter fen hydrology (Cramer 2005), the perennially running water may have flushed out any additional nutrients that were not immediately immobilized by microbial action and rapidly growing ephemeral forb and graminoid species.

If the Biscuit Fire altered soil N in the upland areas, it was likely driven by the dramatic loss of aboveground organic materials (i.e. overstory vegetation, duff, and downed wood; USDA Forest Service and BLM 2004) and the increase in erosion that would follow (Grier 1975, Debano and Conrad 1978, Vitousek and Howarth 1991). The radical alteration of soil physical properties that generally occurs following fire (McNabb and Cromack 1990), coupled with increased erosion with the onset of fall rains (generally between 121 and 200 cm of rain falls in the non-summer months; Borine 1983, Mills and Roath 1984), would drastically alter soil biological processes (Raison 1979). The destruction of the overstory essentially halted any N sequestration by vegetation, meaning there was little plant uptake of the recently increased soil inorganic N pools that result due to rapid microbial action following fire (Raison 19979). Additionally, there may have been a loss of any mycorrhizal associations (Amaranthus and Trappe 1993) and damage to the seed bank (Turner et al. 1994). These changes in the biological characteristics of the soil, in addition to the general infertility and toxicity inherent in serpentine soils, likely prevented rapid post fire recovery by vegetation which would, in turn, lead to decreased overall soil N availability in comparison to unburned upland habitats.
Had I solely utilized soil core sampling to estimate soil N, I would likely have concluded that there was no difference in N availability between the burned and unburned sites. The most likely explanation is that these systems have recovered in the five years since the fire. The soil profile is generally shallow and well drained, meaning that water (and any labile N dissolved in it) would move relatively easily through and out of the system. My study areas experience relatively high rainfall in the winter, thus five seasons of flushing could have restored the soil N dynamics in the burned areas to resemble the unburned areas. Additionally, due to the low ambient soil N, any increase in N from the Biscuit Fire may have been taken up by the vegetation immediately following the fire and sequestered in plant material (Keeley et al. 1981, Boerner 1982).

If the Biscuit Fire had any effect on soil N, it likely affected the two ecosystems differently. This idiosyncrasy was noted by Ratchford et al. (2005) in terms of the effect of the Biscuit Fire on ant assemblages at many of the same fen-upland pairs that I used. They found that the effect of fire on ant assemblages depended on habitat type, and that fire affected ant assemblages inversely in fens and uplands. That is, burned sites were less similar in subsequent years to each other than unburned sites, and fen sites were generally more similar to each other over time than upland sites. Thus, it is likely that any interpretation of the role of fire in a serpentine landscape must pay careful attention to the habitats affected, as not all habitats are affected equally by fire.
Differences in Soil N Among Serpentine Habitats

Perhaps one of the most surprising findings of my work is that no difference in soil N was detected between fen and upland areas in terms of soil NH$_4^+$-N or NO$_3^-$-N by either method replicated in both habitats (buried IER bag and soil cores). These two ecosystems have long been described as distinct vegetation types (Whittaker 1954, Whittaker 1960, DellaSala et al. 1999), but my study indicates they are similar in terms of overall soil N availability. The vegetation difference is therefore likely driven primarily by moisture differences, successional gradients brought on by fires, or the dynamics of other limiting nutrients, and not soil N levels.

Non-woody vegetation is sparse in the uplands (Whittaker 1960), but omnipresent in the fen areas, with a permanent litter layer as well as old *D. californica* pitchers from previous years (D. Franklin pers. obs.). The reducing conditions of the fen soils (Borine 1983, Mills and Roath 1984), coupled with evidence of microbial action (strong sulfurous odor, D. Franklin pers. obs.) and the presence of partially decomposed organic matter (also known as muck), indicates that this litter represents a constant influx of decomposing material. As it is broken down by microbial action, the liberated inorganic N would compensate for the nutrient loss from erosion and leaching due to the high water table. The uplands, however, are almost barren of litter. The lack of available decomposable organic substrate, as well as low moisture levels, would suggest that the uplands are experiencing lower levels of N transformations than the fens. This is confirmed by the low levels of N mineralization and nitrification that I measured, as well as by the lack of organic soil development in any of the uplands that I visited. While
overall N availability did not differ significantly between the two habitats, this is likely the net result of two different N cycling regimes, and not a ubiquitous characteristic of the landscape.

The two ecosystems may differ in their sources of N, but the overall ambient levels remain the same. One would need to pursue a study using stable isotopes (that is, $^{15}$N methods) or quantum dot labeling methods (Whiteside et al. 2009) to reveal the true sources of N in each of these ecosystems, as the superficial analyses I used suggest that net N levels do not vary across the landscape.

Studies of N dynamics in discrete wetland or terrestrial ecosystems are common, but comparative studies of N availability or transformations between wet and dry ecosystems within a single landscape are very rare (for examples, see Zak and Grigal 1991, Axt and Walbridge 1999, Craft and Chiang 2002). In the few studies that have been done, the general finding is that wetlands harbor higher levels of N, especially NO$_3^-$-N. It is worth noting that these studies occurred in drastically different wetland types from the ones I studied (Zak and Grigal 1991, Craft and Chiang 2002), or quantifying N was not the primary goal of the study (Axt and Walbridge 1999). Further comparative studies are necessary in order to understand this wetland-upland dynamic. By sampling towards the end of the growing season, I likely failed to capture the highest amount of N fluctuations in these ecosystems. A study carried out during the spring or fall might report drastically different results.
Lysimeter Method

The resin based lysimeter is a novel approach to deploying IER bags in a wetland habitat, and appeared to represent soil N dynamics that are more similar to a soil core than to the buried IER bag. In fact, there was no statistical difference between N availability estimates between this method and direct soil sampling. The resin based lysimeter seemed to provide a more accurate snapshot of conditions specific to collection time in a wetland ecosystem, rather than the longer-term picture provided by the IER bag buried directly in the soil. Additionally, IER bags monitor some level of biological activity, and removing them from direct soil contact may minimize this sampling bias.

There are two main benefits to using the resin based lysimeter method; first, the IER bag is not directly exposed to the soil surface, but rather to groundwater, so one develops a better understanding of nutrient dynamics and how nutrients are moving through the ecosystem, rather than simply soil N availability. Additionally, by sampling the same location through time, a more precise measure of the nature of the wetland may be obtained, where in a bigger application or installation, one could even monitor both temporal as well as spatial N dynamics over a large wetland area. Due to limited sample size and number of replicates, coefficients of variation (CV) could not be used for formal statistical comparisons, however the buried IER bag method had a CV 2.5 times higher than the soil core (0.633 vs. 0.279), whereas the lysimeter method had a CV closer to 1.75 times that of the soil core (0.466). The lysimeter method may control for some of the variation inherent in soils by sampling the subsurface flows rather than the soils themselves. As fens represent one of the most threatened habitat types (Frost and Jules
in this landscape, the resin based lysimeter provides a way to monitor soil nutrient dynamics with a minimal of disturbance, and is therefore ideal for long term studies. Further exploration and development of this method in other wetland habitats is required.

Comparisons to Previous Research

My research represents the first study to take a detailed look at the effect of fires on serpentine soil N availability, and the first to look specifically at *D. californica* fens in relation to surrounding upland areas. The only other study on fire and N on serpentines only looked at gross N as part of a bigger investigation into the nature of community diversity (Safford and Harrison 2004). Safford and Harrison (2004) found that soil N played a significant role in postfire vegetation recovery, they did not detect a difference between burned and unburned plots using a soil sampling protocol similar to mine. However, they were only concerned with total soil N, which incorporates both the inorganic forms of N that I measured, and organic forms that are not available to plants, and effectively measures a different metric of soil fertility.

The values I obtained for soil N availability are much lower than other studies that have been done on serpentine grasslands (Christensen and Muller 1975, Turitzin 1982, Reynolds et al. 1997), although these have generally occurred south of my sites. Reynolds et al. (1997) studied serpentine grasslands at the Jasper Ridge Biological Preserve in Stanford, Ca. and detected almost 170 times as much NO$_3^-$-N in the soil using IER bags compared to the serpentine soils of southwestern Oregon and northern California. However, Huenneke et al. (1990) worked in Santa Clara County, Ca, and
reported higher NH$_4^+$-N concentrations but similar NO$_3^-$-N concentrations in the soil. Despite these conflicting results, the overall consensus is that these soils are N limited, although the degree of limitation may vary by substrate mineral composition and climate. For comparison, a study conducted in *Pinus ponderosa* stands in Deschutes National Forest (approximately 285 km northeast of my study sites) reported NO$_3^-$-N values approximately 1000 times greater than values I detected. And a typical N saturated eastern *Tsuga canadensis* forest generally will generally have between 1000 and 8000 times more N available to plants than serpentine ecosystems (D. Franklin, *unpub*).

Conclusions and Recommendations

It is worth noting that any findings on the effect of fire on N dynamics from a five year post-burn perspective would be at least somewhat anecdotal due to the lack of pre-burn data. However, other findings have reported on detectable effects of fire for longer time frames (e.g. up to 12 years, Monleon et al. 1997) without pre-burn data. Additionally, I had a relatively low sample size, which made drawing strong conclusions difficult.

While this study represents the first foray into understanding soil N in this landscape, it also illustrates that there are significant questions still to be answered in terms of N availability in a heterogeneous serpentine landscape, the effect of fire in these ecosystems, as well as the ability of the various N quantification methods to accurately portray soil N in a severely N limited habitat. Further studies should increase their replication, as well as track soil N year round. Due to time and budget constraints, as
well as significant safety concerns due to the remoteness of some of these habitats, no attempt was made to gain an understanding of year-round N dynamics. Soil N may fluctuate more in other season, but I can only comment on the soil conditions in the peak of summer.

The interpretation of the role of fire in these ecosystems varies depending on what method one uses to quantify N. Future studies should compare soils over a more extensive sample of habitat coupled with laboratory incubations to estimate maximum N transformation rates. Other aspects of ecosystem function and characteristics should also be taken into account in future studies. Decomposition was not quantified in the fens; however plant litter decomposition is thought to play a significant role in understanding N cycling in wetland environments as it represents the one of the most significant inputs of N into these systems (Bowden 1987). Additionally, fertilization experiments (either in situ or in a greenhouse) would give greater insight as to the true limiting nature of these soils and may elucidate the some of the complexities of limiting nutrients in these soils. Although N is thought to be an important limiting factor to plant growth in serpentine habitats, there is also literature suggesting that phosphorus may be equally limiting (Turitzin 1982, Huenneke et al. 1990). Due to an unidentified precipitate in the tubes of the autoanalyzer, P was not measured, but future studies should take it into consideration in both study design as well as laboratory methods. There is much work remaining to do in unraveling the Gordian knot of what exactly controls plant growth and community structure in terms of soil characteristics and N dynamics in serpentine ecosystems.
LITERATURE CITED


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SPSS. 2008. SPSS version 17.0. SPSS, Chicago, Illinois, USA.


