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From Seed to Sky: Impacts of explosive compounds on vegetation across spatial and developmental scales

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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Abstract

FROM SEED TO SKY: IMPACTS OF EXPLOSIVE COMPOUNDS ON VEGETATION ACROSS SPATIAL AND DEVELOPMENTAL SCALES

By Stephen M. Via Ph.D.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Explosive compounds are broadly distributed across the globe as a result of nearly two centuries of munitions use in warfare and military activities. Two explosive compounds have seen disproportionate use; RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and TNT (2-methyl-1,3,5-trinitrobenzene), being the most commonly found explosives in the environment. The effects of explosives on biota have been studied in great detail; however, there is a general lack of understanding with regard to broader ecological impacts of these contaminants. My dissertation objective was to follow the impacts of explosive compounds on vegetation across scales. Impacts on vegetation at the species scale alter community composition via species-

specific and age-specific responses to explosives. Results presented here showed that contaminated soils induced a variety of responses in vegetation, yet impacts to water relations were similar regardless of species. Use of novel metrics in monitoring plant responses to explosives compounds aided in delineation of reference and treatment groups. At the community scale the presence of explosives induced species and functional composition shifts. The observed shifts are likely due to physiological impairment as individuals in the field exhibited significant impacts to physiological functions. Effects of explosives contamination also detectable using remote sensing techniques. Impacts to plant morphology and physiology are directly related to community level shifts observed in long contaminated areas. This highlights the long lasting impacts that these largely overlooked contaminants can have on a system and opens avenues for new, at range, vegetation based contaminant detection systems.

Introduction

Through a combination of perpetual industrial activity and large scale warfare in the past centuries, explosives have been deposited into soils all around the globe (Pichtel, 2012). Although there is a thorough understanding of the impacts of explosives on human health, there is very little knowledge of ecological impacts of these contaminants from field based studies. A large amount of what is understood comes from the extrapolation of small scale laboratory findings and therefore must be considered tentative estimates at best (Via and Zinnert, 2016). Many factors present in the field cannot be replicated or accounted for in a laboratory setting. Laboratory studies are essential to understanding the responses of biota to explosive compounds, especially given the highly variable nature of their impacts (Pilon-Smits, 2005). Explosive compounds have different impacts on biota, and those impacts can further vary based on species, age of individuals, concentration of contaminant, length of exposure time, and surrounding environmental and climactic conditions (Pennington and Brannon, 2002; Pilon-Smits, 2005; Via and Zinnert, 2016). Given these considerations, field based studies are essential in furthering our understanding of the larger ecological impacts that these contaminants can have.

Plant-explosive interactions, in particular, have been studied in great detail (reviewed in Via and Zinnert, 2016), largely for the purpose of using vegetation as a means to decontaminate degraded locations (Pilon-Smits, 2005; Kiiskila *et al.*, 2015). This focus has expanded the understanding of explosive uptake, sequestration, metabolism, and morphological responses with

regard to vegetation (Best *et al.*, 2007), but ignored the physiological impacts which can also occur (Naumann *et al.*, 2010, Zinnert, 2012; Via *et al.*, 2014). Physiological responses are indicative of cellular process, impairment or change, and ultimately are the cause of observed differences in well-studied metrics such as growth (Lambers *et al.*, 2008).

Small scale impacts of explosives on the health of individual species can have far-reaching implications for larger scale community and ecosystem function and stability (Walker and Wardle, 2014). The presence of explosive compounds acts as a physiological filter controlling species establishment and success in contaminated areas. Inducing changes in species presence or abundance can have drastic impacts on overall community and ecological health (Hooper *et al.*, 2005). Thus a cross-scale approach is needed to reduce reliance on speculation.

My overall objective was to investigate and develop linkages between the impacts of explosives across spatial and developmental scales. Specific goals were carried out in four parts: 1) compile the existing literature to fully understand the current state of plant-explosives interaction work and develop a linkage framework for cross-scale comparisons, 2) investigate the physiological and morphological impacts of explosive compounds on various plant species, 3) quantify impacts of explosive compounds on plant community species and functional composition, richness, and diversity, and 4) investigate the potential to detect the presence of explosive compounds via remote sensing of vegetation in the field.

Chapter 1

Impacts of explosive compounds on vegetation: A need for community scale investigations

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Introduction

Due to long-term and widespread use of munitions for both military and civilian purposes, explosive compounds contaminate large portions of most continents (Pichtel, 2012). Research examining the impacts of explosives on vegetation has been ongoing for over 20 years, enhancing our understanding of toxicity (Best *et al.*, 2006; Best *et al.* 2008; Vila *et al.*, 2007a; Vila *et al.*, 2007b; Vila *et al.*, 2008) and ability to remediate contaminated sites (Pilon-Smits, 2005; Singh and Mishra, 2014; Kiiskala *et al.*, 2015). While not the focus of past research, the literature suggests significant and lasting ecological impacts from this increased presence of explosives. Ecological studies have investigated similar effects of other anthropogenic disturbances ranging from agrochemicals (Coutris *et al.*, 201; Halstead *et al.*, 2014), mine tailings (Wang *et al.*, 2010; Donggan *et al.*, 2011; Pandey *et al.*, 2014), heavy metals (Baruttia *et al.*, 2011; Perrino *et al.*, 2014), and radioactive waste (Woodwell and Sparrow 1963; Woodwell and Oosting 1965). As with other contaminants, explosive compounds can influence ecological and environmental processes. Munitions (termed unexploded ordnances or UXOs) which are lost, buried, undetonated, or partially detonated pose a greater ecological threat than those which properly detonate or are handled correctly (Pichtel, 2012; Taylor *et al.*, 2015). The most commonly used and studied explosives are RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and TNT (2-methyl-1,3,5-trinitrobenzene; Hawari *et al.*, 2000; Rylott and Bruce, 2009; Anerdon, 2010; Khatisashvili *et al.*, 2009) and will be the focus of this review. In this review, we synthesize the literature regarding effects of RDX and TNT on plants and provide an ecologically relevant conceptual framework as there is need for a community scale focus.

Studies regarding explosives and vegetation include uptake ability, germination inhibition, morphological responses, trophic transfer potential, physiological responses,

compound degradation, transformation processes, and genotoxicity. Laboratory experiments are essential for investigating specific responses, but it is difficult to translate knowledge acquired in the lab to use in field settings (Hawari *et al.*, 2000; Kiiskila *et al.*, 2015). Due to inherent limitations and hazards, field studies focused on explosives are few (Travis *et al.*, 2008) with the majority emphasizing phytoremediation (see Hawari *et al.*, 2000 and Pilon-Smits, 2005 for review), leaving a gap in our understanding of long-term effects of explosives in the environment of these concepts across both spatial and temporal scales.

Effects of explosives on vegetation at the individual scale occur relatively rapidly (Krishnan *et al.*, 2000; Winfield *et al.*, 2004; Best *et al.*, 2006; Vila *et al.*, 2005; Vila *et al.*, 2007a, Vila *et al.*, 2007b, Vila *et al.*, 2008, Naumann *et al.*, 2010, Ali *et al.*, 2014; Via *et al.*, 2014, and Via *et al.*, 2015) and vary based on a number of factors, ranging from species to soil type (Scheidemann *et al.*, 1998; Price *et al.*, 2002; Winfield *et al.*, 2004; Kiiskila *et al.*, 2015). Impacts to vegetation can be direct, via toxic effects to plant tissues (Best *et al.*, 2006; Vila *et al.*, 2005; Vila *et al.*, 2007a, Vila *et al.*, 2007b, and Vila *et al.*, 2008), or indirect, via impacts to microbial communities (Thijs *et al.*, 2014). Regardless of pathway, explosives can limit the ability of vegetation to colonize, expand, reproduce, and grow in contaminated areas, acting as a physiological filter and shaping standing communities over the long-term (Lambers *et al.*, 2008). Alterations of the community can further influence ecosystem function and overall health. Thus, effects of explosives on vegetation bridge both spatial (individual to ecosystem) and temporal (hours/days to centuries or millennia) scales (Figure 1.1). Research examining interactions of responses across scales can aid in prediction of explosives impact on communities and ultimately ecosystem function. We propose that through connecting individual response data directly to the

large scale impacts of explosives provides quantitative relationships and a framework for more accurate extrapolation of fine-scale response data from previous studies.

Explosives in the environment

Explosives have civilian, industrial, and military uses resulting in varied sources of contamination (Myler and Sisk 1991; Pichtel, 2012; Kholodenko *et al.*, 2014); however, the largest contributor of explosives into the environment are military activities and associated industries (Best *et al.*, 1999; Just and Schnoor, 2004; Pichtel, 2012; Certini *et al.*, 2013). Globally, 68 nations have declared a munitions issue within borders (Figure 1.2; The Monitor, 2009) as a direct result of current and past conflicts. During World War II (WWII), 2-2.7 million tons of bombs were dropped on Germany and occupied Europe. With a known failure rate ranging between 5 and 15% (Eckardt, 2012) there are 27,000 –300,000 UXOs across Europe today (Abad-Santos 2012). Germany has ~ 391,000 ha still in need of bomb removal (Crossland, 2008) with more than 3,000 bombs suspected to be in the soil in Berlin (Huggler, 2015), and ~2,500 bombs in Munich (Abad-Santos 2012). The Korean War left 9100 ha of land outside the demilitarized zone (DMZ) which are known to be mined (The Monitor, 2009). Laos contains 750,000 tons (roughly 80 million individual pieces; UXO LAO, 2013), of ordnance in its soils (Suthinithet 2010 and Pichtel, 2012). Iraq has accumulated ~20 million landmines since the 1940s, covering ~150 million ha (CISR 2013). Due to past engagements, the Syria-Turkey border is covered with between 613,000 and 715,000 landmines, (HRW, 2014). UXOs are difficult to detect and dangerous to remove, posing a long-term threat both in explosive potential of the ordnance as well as toxicological threat of the compounds.

Munitions and their associated contaminants are not solely found within the confines of battlefields, but are present on military bases, bombing ranges, artillery firing ranges, as well as

industrial sites (Figure 1.2; Pichtel, 2012 and Taylor *et al.*, 2015). In the United States there are roughly 2000 Department of Defense locations with explosives contaminated soils and numerous Environmental Protection Agency Superfund sites that include explosives among their lists of contaminants (EPA 2014).

Once released into the environment, explosive compounds do not remain in a static location but are mobile in the soil pore matrix and radiate out from the contaminant source (Pennington and Brannon, 2002 and Kiiskila *et al.*, 2015). Concentrations of explosives in soil vary depending on source, environment, and surrounding biota. Soil concentrations for RDX range from 0.7 to 74,000 mg kg⁻¹ (ppm) dry soil and TNT from 0.08 to 87,000 mg kg⁻¹ (ppm) (Best *et al.*, 2008; Best *et al.*, 2009). The degree of variability in contaminant concentration represents a large hurdle to accurately predict ecological impacts of explosives. Once the concentration or a site is known certain characteristics of the compounds present can be used to predict their behavior in the soil. Mobility and absorptivity of explosives in soil can be estimated using the octanol-water partition coefficient (K_{ow}). This acts as an indicator of the potential for a compound to adsorb to soil; compounds with high K_{ow} have high hydrophobicity and high binding with soils (USGS, 2014). RDX and TNT have K_{ow} values of 0.87 and 1.6, respectively; thus, RDX less likely to bind to soil particles relative to TNT (Yoon *et al.*, 2005; Singh and Mishra, 2014). Organics with $K_{ow} < 3$ (i.e. RDX and TNT) migrate in soil pores via water and are available for plant uptake (Talmage *et al.*, 1999 and Larson *et al.*, 2008).

Soil types where munitions are present play a large role in migration and bioavailability of explosive compounds (Pennington and Brannon, 2002 and Monteil-Rivera *et al.*, 2009). Clay soils adsorb more explosive compounds than sandy soils (Larson *et al.*, 2008 and Vierling and Kimpel, 1992) and humic soils bind more TNT than other soil types (Pilon-Smits, 2005). As

organic material in soil decreases, so does binding potential, increasing bioavailability of explosive compounds (Vierling and Kimpel, 1992). Soil characteristics are important when considering the behavior of released explosives, yet a large portion of the binding, transformation, and degradation observed in soils is due to microbial activity. The ability of microorganisms to degrade or transform explosives can be limited by a number of factors including nutrient, water, carbon, and oxygen availability (Wenzel, 2009). Plant roots release photosynthetically derived compounds, water, and oxygen into the surrounding soil producing an area of increased microbial activity, in the rhizosphere and subsequent degradation of explosives (Pilon-Smits, 2005; Wenzel, 2009; Strand *et al.*, 2014). The extent of biodegradation that occurs in the rhizosphere determines mobility and bioavailability of explosives. This interplay between plants, rhizosphere microbial communities, and soil characteristics is important when considering impacts of explosives on vegetation. Given spatial heterogeneity of contamination and broad range of possible environmental conditions, a thorough understanding of edaphic characteristics at each location is required to predict how explosives will interact with vegetation.

Plant uptake of explosives

Explosive compounds are not naturally occurring and there is no natural method of transport to move explosives through cellular membranes (Pilon-Smits, 2005). Interaction of explosive compounds and root surfaces determine uptake while the tendency of organic pollutants to move into plant roots is controlled by characteristics of the compound, soil, and plant (Paterson *et al.*, 1990; Pilon-Smits, 2005). Because explosives readily enter the soil solution, they may enter plant roots unimpeded via between membrane spaces associated with apoplastic transport (Ghosh and Singh, 2005) due to bulk flow water movement driven by transpiration (Singh and Mishra, 2014). Once inside the root explosives move through the

protective Casparian strip, enter the xylem, and are distributed throughout the plant (Pilon-Smits, 2005).

Inside plants, organic compounds undergo three distinct phases: transformation, conjugation via chelators, and sequestration; described as the “green liver model” (Klein and Scheunert, 1982; Sandermann, 1994; Burken and Schnoor, 1997; Hannink *et al.*, 2002). Explosives tend to follow this model and undergo enzymatic transformation and conjugation with D-glucose, amino acids, or glutathione into either soluble or insoluble products (Sens *et al.*, 1999; Robidoux *et al.*, 2003; Vila *et al.*, 2008). Soluble products can be either stored in the vacuole or in the cell wall, while insoluble products go directly to the cell wall (Burken *et al.*, 2000; Lotufo *et al.*, 2009; Rylott *et al.*, 2011). Both RDX and TNT are commonly bound to lignin, cellulose, and within vacuoles. Chemical structure plays an integral role in the behavior of the compound once in the plant (Burken *et al.*, 2000); RDX is primarily stored in leaf tissues, while TNT is stored almost exclusively in root tissues (Figure 1.3; Vila *et al.*, 2007a; Vila *et al.*, 2008). Interestingly these patterns of contamination partitioning are conserved across species and yet impacts on plant health and function are not (Table 1.1).

Both compounds are phytotoxic in parent form as are many of the compounds produced via degradation. Degradation of RDX and TNT can produce amino derivatives as well as a variety of further reduced compounds including, oxygen radicals, formaldehyde, nitrous oxide, and carbon monoxide (Spain, 1995; Hawari *et al.*, 2000; Halasz *et al.*, 2002; Bernstein and Ronen, 2012). Impacts range from shifts in cellular function to visible morphological deformation, which vary based on a range of factors such as species, age of individuals, compound type, and concentration (Robidoux *et al.*, 2003; Winfield *et al.*, 2004; Just and Schnoor, 2004; Vila *et al.*, 2008, Ali *et al.*, 2014; Via *et al.*, 2014).

Morphological and physiological impacts

Morphological impacts of RDX and TNT reflect the localization of the compounds in the plant. RDX causes significant damage to aboveground portions of plants (Vila *et al.*, 2007b; Khatisashvili *et al.*, 2009; Singh and Mishra, 2014), while TNT damages the belowground tissues (Peterson *et al.*, 1998; Gong *et al.*, 1999; Krishnan *et al.* 2000; Vila *et al.*, 2007a; Khatisashvili *et al.*, 2009; Singh and Mishra, 2014). In general, plants exhibit negative responses to TNT at much lower concentrations (e.g. 30-60 ppm) than for RDX (e.g. 100-500 ppm; Peterson *et al.*, 1996; Peterson *et al.*, 1998; Gong *et al.*, 1999; Pilon-Smits, 2005; Vila *et al.*, 2005; Zinnert, 2012; Via *et al.*, 2014). The most common response to RDX is leaf necrosis, starting at leaf margins and moving inward over time. RDX also causes curled or irregular leaf margin, fused leaves, bifurcated leaves, atypical pigmentation, reduced shoot length, decreased leaf expansion, delayed emergence, and atypical bilateral symmetry (Winfield *et al.*, 2004; Vila *et al.*, 2007b; Via *et al.*, 2014). Less common responses include thin stems, underdeveloped roots, reduced root length, curled root tips, and decreased root exudates (Sens *et al.*, 1999; Winfield *et al.*, 2004; Vila *et al.*, 2007b). In contrast, TNT can inhibit growth of new roots and root hairs in addition to destroying the roots and hairs that are present at the time of exposure (Palazzo and Leggett, 1986; Peterson *et al.*, 1998; Krishnan *et al.*, 2000; Gong *et al.*, 1999). Despite many studies quantifying morphological impacts at various concentrations of explosive compounds on different plant species, only a few studies have investigated physiological impacts (Thompson *et al.*, 1998a, Ali *et al.*, 2006; Zinnert, 2012; Zinnert *et al.*, 2012; Via *et al.*, 2014).

Similar to morphological impacts, physiological responses to TNT are present at much lower concentrations than for RDX (Peterson *et al.*, 1996; Peterson *et al.*, 1998; Pilon-Smits, 2005; Vila *et al.*, 2005; Zinnert, 2012; Via *et al.*, 2014). This may be due to TNT sequestered in

roots affecting growth and function through alterations of water and nutrient uptake (Sens *et al.*, 1999; Winfield *et al.*, 2004; Vila *et al.*, 2007a), leading to drastic impacts on plant health (Ploetz and Schaffer, 1989). Stress tends to activate similar physiological response pathways in vegetation regardless of the stress source (Chapin III 1991); however, explosive compounds do not always follow this pattern. For example, woody species exposed to TNT and RDX do not exhibit typical curvilinear and highly related responses in stomatal conductance and photosynthesis normally found under natural stress (Flexas *et al.*, 1999; Zinnert *et al.*, 2012; Via *et al.*, 2014). This may be due to a decoupling of physiological processes which are tightly linked under normal conditions. In particular, TNT appears to impact photosystem II (PSII) as evidenced by electron transport rate (ETR) and dark minimal fluorescence (F_0) (Ali *et al.*, 2006) while RDX appears to impact the light-independent portion of photosynthesis (Via *et al.*, 2014). Despite commonalities among plant species morphological and physiological responses to explosives there is variation in the degree of response present. This variation is further complicated as individual species show different responses to explosives across life stages.

Impacts on life stage

Plant life stages are typically classified as seed, seedling or juvenile, and adult. The seed stage refers to the pre-emergence embryonic stage, the seedling stage refers to post emergence individuals still pulling energy from their cotyledons, juveniles are individuals who have stopped depending on their cotyledons but are not yet large or old enough to reproduce, and any individual capable of reproducing is counted as an adult. As plants age, biochemical processes change (Bond, 2000; Donaldson *et al.*, 2006; Juvany *et al.*, 2013) and lead to different stress responses at each life stage. Seeds mechanically absorb contaminants as they imbibe water to initiate germination (Rajjou *et al.*, 2012) while juvenile and adult life stages both pull

contaminants from the soil matrix along with water moving in the soil-plant-atmosphere continuum (Pilon-Smits, 2005). Mature plants have more biomass relative to earlier life stages, which allows plants to withstand stress associated with explosive compounds by accumulating the compounds into older leaves, senescing those leaves, and subsequently regrowing healthy foliage (Via *et al.*, 2014). The commonly accepted pattern of toxin response in plants is that juveniles are most sensitive and sensitivity declines with age (Niinemets, 2010). Interestingly, plant responses to explosive compounds vary by species; some appear unaffected by RDX or TNT at any life stage, and others suffer significant impacts in one life stage but not another (Peterson *et al.*, 1998; Krishnan *et al.*, 2000).

Successful germination does not predict successful growth in the presence of contamination (Gong *et al.*, 1999). Smooth brome grass (*Bromus* sp.), *Andropogon geraldii*, *Bouteloua gracilis*, and switchgrass (*Panicum virgatum*) all successfully germinate in the presence of TNT but become less tolerant as they age, showing significant reductions in health at similar concentrations to when germination occurred (Peterson *et al.*, 1998; Krishnan *et al.*, 2000; Best *et al.*, 2007). Some species, such as *Sorghastrum nutans*, *Portulaca oleracea*, and *Sida spinosa*, have the opposite response in the presence of explosives where germination is reduced but growth is stimulated at certain contamination levels (Best *et al.*, 2007). Via *et al.*, (2014; 2015) showed that for the shrub *Morella cerifera*, germination was not affected by TNT (up to 900 ppm) while adult physiology was significantly impaired at much lower concentrations (~30 ppm TNT, ~100 ppm RDX). However, *M. cerifera* plants exposed to RDX showed significant impacts at all life stages tested. With such a range in responses across life stages, more studies are needed comparing within and among functional groups and that follow

individual plants from germination through establishment to fully understand plant tolerance and associated mechanisms (Peterson *et al.*, 1998).

RDX or TNT alone can have significant impacts on vegetation, but the combination of the two may have synergistic, additive, or conflicting impacts (Best *et al* 2007; Best *et al* 2009; Panz *et al.*, 2013; Ali *et al* 2014; Via *et al* 2014). Comp B, due to its wide historical use, is one of the more common munitions mixtures found in the environment. Simultaneous interaction of RDX and TNT produces responses which are similar to those induced by either alone but are still distinguishable. For example, *Morella cerifera*, when exposed to RDX or TNT, exhibited reductions in both physiology and morphology; however individuals exposed to Comp B exhibited mixed responses (Via *et al.*, 2014).

The consensus thus far is that there are varying degrees of plant responses with each life stage when exposed to explosive compounds (Table 1.2). This inconsistency of response demonstrates that vegetation exhibits a range of contaminant tolerance. Differences in plant responses influence vegetation establishment and persistence in the field and alter community composition. Therefore, presence of contaminants is not only problematic for individual species or taxonomic groups, but for surrounding ecological communities and the overall ecosystem.

Community level impacts

Community level shifts have been documented for a range of anthropogenic disturbances and contaminant releases (Prach, *et al.*, 2014) from mining operations (Brady and Noske 2010, Zhang and Chu, 2011; Levy and Cumming, 2014; Pandey *et al.*, 2014) to radiation exposure (Woodwell 1963, Woodwell and Sparrow 1963, Woodwell and Rebeck, 1967, and Woodwell and Whittaker 1968). Explosive compounds have the potential to act as physiological filters

suppressing intolerant species and clearing space for tolerant ones, altering plant establishment and thereby changing community composition (Newman and Clements, 2007 and Sulmon *et al.*, 2015; Figure 1.4). Physiological filters can alter species richness, composition, and overall community structure (Bazzaz, 1979) by suppressing the presence of some species while promoting the occurrence of others. While modifying species composition of an area does not necessarily indicate that the system will be impaired there are other effects of explosives which alter community function and resilience. Changes caused by explosives occur across species, genera, and functional types. For example, in contaminated and disturbed areas, annuals are more common than perennials (Schnoor, *et al.*, 1995; Quist *et al.*, 2003; Zhang and Chu, 2013), monocots are more tolerant of explosives than dicots (Winfield *et al.*, 2004 and Panz *et al.*, 2013), and the responses seen in woody species are more substantial than those in herbaceous and vine species (Via, unpublished data). While new species can fill gaps left by extirpated ones, removing an entire functional group, and subsequently preventing others of that group from establishing, has the potential for more drastic impacts (Tilman *et al.*, 1997).

Such changes have not only been documented in standing cover but also in the seedbank in contaminated areas (Zhang and Chu, 2013; Via, unpublished data), further preventing community recovery. In many cases distribution of explosive compounds is accompanied by some form of disturbance (i.e. munitions detonation); presence of explosive compounds may influence succession and revegetation of disturbed and contaminated areas (Zhang and Chu, 2011 and Belote *et al.*, 2012). Further, the stability and recalcitrant nature of these compounds in the environment (Hannink *et al.*, 2002; Panz and Miksch, 2013) add to the complexity of community level responses. Long lasting impacts and a persistent nature suggest that explosives can have long-term influences on vegetation.

Given the amount of research on explosives-vegetation interactions, there is still much unknown about environmental impacts. Relatively few non-agronomic, non-transgenic, naturally occurring species have been subjected to quantifiable analysis of explosive compounds (for review see: Panz and Miksch, 2012). Most studies have focused on RDX or TNT (Peterson *et al.*, 1998; Sens *et al.*, 1999; Thompson *et al.*, 1998a, Winfield *et al.*, 2004; Ali *et al.*, 2006; Vila *et al.*, 2007a, Khatisashvili *et al.*, 2009; Zinnert, 2012; Zinnert *et al.*, 2012; Singh and Mishra, 2014); comparatively few have investigated the impacts of mixtures, such as Comp B (Best *et al.*, 2009; Ali *et al.*, 2014; Via *et al.*, 2014). Without a solid understanding of the effects explosives on species which naturally colonize contaminated areas, it is difficult to predict impacts of these contaminants in the field. This is vital as explosives can have varied effects on vegetation crossing multiple scales of time and space.

Summary

Known responses of dozens of plant species in various growing media, contaminant concentration, and life stage to explosives contamination provide a strong foundation to build and expand our knowledge of explosives impacts on the environment. Explosives significantly impact vegetation morphology and physiology (Thompson *et al.*, 1998a, Sens *et al.*, 1999; Winfield *et al.*, 2004; Ali *et al.*, 2006; Khatisashvili *et al.*, 2009; Zinnert, 2012; Zinnert *et al.*, 2012; Singh and Mishra, 2014; Via *et al.*, 2014). This limits the potential of many species to inhabit contaminated areas (Hooper *et al.*, 2005). Through a broadening of the current literature to include physiological, morphological, and reproductive impacts for species and functional groups, a full understanding of the small scale impacts of explosives can be readily obtained. With a stronger base of knowledge, extrapolations and inferences can be made towards understanding the community scale.

It is well established that anthropogenic impacts alter community composition and impact species diversity (Hautier *et al.*, 2015; Sulmon *et al.*, 2015), and that functional trait distribution represents legacy effects of species filters (Vandewalle *et al.*, 2014). This has significant implications for common and foundational species, the loss of which can have broad consequences for community function, stability, and structure (Tilman *et al.*, 1997; Ellison *et al.*, 2005; Tilman *et al.*, 2014). Specifically these shifts translate into modification of ecosystem water balance (Ellison *et al.*, 2005), nutrient cycling, microclimate (Tilman *et al.*, 1997 and Ellison *et al.*, 2005), primary productivity (Tilman *et al.*, 1997), stability (Hautier *et al.*, 2015; Tilman *et al.*, 2014), and invasion potential (Tilman *et al.*, 2014). The impacts reach well beyond the plant community in that diversity loss at one trophic level leads to cascades of change in others (Tilman *et al.*, 2014) implicating all biota reliant on vegetation. This is compounded by the fact that these contaminants are mobile (Pennington and Brannon, 2002), recalcitrant (Robidoux *et al.*, 2009), highly toxic (Best *et al.*, 2006; Best *et al.*, 2008, Vila *et al.*, 2007a; Vila *et al.*, 2007b), and have the potential to bioaccumulate and biomagnify in both vegetation and animals (Johnson *et al.*, 2009).

To extrapolate beyond the individual species response, it is essential to expand the current understanding of individual or species level impacts (morphology and physiology) of explosives to include native species that naturally occur in impacted areas, and include studies that focus on community level impacts. By further investigating small-scale responses more accurate predictions of species or taxonomic group responses to explosive compounds will be possible. Through scaling up, we can quantify impacts of explosives on larger scales and test our ability to accurately use small-scale data, which are readily available, in predicting the larger scale responses (Halstead *et al.*, 2014; Walker and Wardle 2014).

Table 1.1: Summary of publications investigating the impacts of explosive compounds on vegetation. Observations are grouped based on the contaminant in question (i.e. RDX, TNT, and Composition B). References seen in the right column represent both seminal and more recent publications; it is not a complete list of publications. Species tested are listed alphabetically and grouped by publication. Reference superscripts denote growing media used (^a = soil, ^b = hydroponics, ^c = agar) and life stage tested (¹ = Embryo, ² = Seedling, ³ = Juvenile, ⁴ = Adult).

| Contaminant | Observation | Species | Reference |
|-------------|---|--|---|
| TNT | Accumulation in belowground tissues | <i>Aleopecurus pratensis</i> , <i>Bromus inermis</i> , <i>Festuca rubra</i> , <i>Lolium perenne</i> , <i>Lupinus angustifolius</i> , <i>Medicago sativa</i> , <i>Phacelia seicea</i> , <i>Phaseolus vulgaris</i> , <i>Phleum</i> sp., <i>Trifolium repens</i> , <i>Triticum aestivum</i> ; <i>Populus deltoids</i> , <i>Popsolum notatum</i> | Scheidemann <i>et al.</i> , 1998 ^{a,3} ; Brentner <i>et al.</i> , 2010 ^{b,3} |
| TNT | Growth inhibition at high concentrations (>~30 ppm) | <i>Cyperus esculentus</i> ; <i>Bromus inermis</i> , <i>Popsolum notatum</i> ; <i>Bromus inermis</i> , <i>Festuca arundinacea</i> ; <i>Acena sativa</i> , <i>Brassica rapa</i> , <i>Lepidium sativum</i> , <i>Triticum aestivum</i> ; <i>Cyperus esculentus</i> , <i>Lactuca sativa</i> , <i>Lycopersicon lycopersicum</i> , <i>Raphanus sativus</i> , <i>Zea maize</i> ; <i>Lactuca sativa</i> ; <i>Cicer arietinum</i> , <i>Glycine max</i> , <i>Lathyrus sativum</i> , <i>Lolium multiflorum</i> , <i>Medicago sativa</i> , <i>Vigna sinensis</i> , <i>Vigna radiata</i> , <i>Zea mays</i> | Palazzo and Leggett, 1986 ^{b,4} ; Peterson <i>et al.</i> , 1998 ^{c,1,2} ; Krishnan <i>et al.</i> , 2000 ^{a,1,3} ; Gong <i>et al.</i> , 1999 ^{a,2} ; Price <i>et al.</i> , 2002 ^{a,4} ; Ali <i>et al.</i> , 2006 ^{a,2} ; Khatisashvili <i>et al.</i> , 2009 ^{b,1,2} |
| TNT | Growth promotion at low concentration (<~30 | <i>Acena sativa</i> , <i>Brassica rapa</i> , <i>Lepidium sativum</i> , <i>Triticum aestivum</i> ; <i>Cicer arietinum</i> , <i>Glycine max</i> , <i>Lathyrus sativum</i> , <i>Lolium multiflorum</i> , <i>Medicago</i> | Gong <i>et al.</i> , 1999 ^{a,2} ; Khatisashvili <i>et al.</i> , |

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| | ppm) | <i>sativa, Vigna sinensis, Vigna radiata, Zea mays</i> | 2009 ^{b,1,2} |
| TNT | Significant morphological damage to roots | <i>Cyperus esculentus; Bromus inermus, Popsolum notatum; Bromus inermus, Festuca arundinacea; Acena sativa, Brassica rapa, Lepidium sativum, Triticum aestivum</i> | Palazzo and Leggett, 1986 ^{b,4} ; Peterson <i>et al.</i> , 1998 ^{c,1,2} ; Krishnan <i>et al.</i> , 2000 ^{a,1,3} |
| TNT | Damage to photosynthetic functioning | <i>Lactuca sativa; Morella cerifera</i> | Ali <i>et al.</i> , 2006 ^{a,2} ; Via <i>et al.</i> , 2014 ^{a,4} |
| TNT | Damage to water relations | <i>Morella cerifera; Morella cerifera</i> | Naumann <i>et al.</i> , 2010 ^{a,4} ; Via <i>et al.</i> , 2014 ^{a,4} |
| TNT | Inhibitions germination | <i>Bromus inermus, Popsolum notatum; Bromus inermus, Festuca arundinacea; Acena sativa, Brassica rapa, Lepidium sativum, Triticum aestivum; Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays; Morella cerifera</i> | Peterson <i>et al.</i> , 1998 ^{c,1,2} ; Krishnan <i>et al.</i> , 2000 ^{a,1,3} ; Khatisashvili <i>et al.</i> , 2009 ^{b,1,2} ; Via <i>et al.</i> , 2015 ^{a,1,2} |
| TNT | Dicots more susceptible than monocots | <i>Acena sativa, Brassica rapa, Lepidium sativum, Triticum aestivum</i> | Gong <i>et al.</i> , 1999 ^{a,2} |
| RDX | Dicots more susceptible than monocots | <i>Brassica rapa, Cucumis sativa, Dactylis glomerata, Festuca arundinacea, Helianthus annuus, Lactuca sativa, Lespedeza capitata, Lolium perenne, Onobrychis viciifolia, Poa pratensis, Sanguisorba minor, Trifolium pretense, Trifolium repens, Triticum aestivum, Zea mays</i> | Winfield <i>et al.</i> , 2004 ^{a,1,2} |
| RDX | Accumulation in aboveground tissues | <i>Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maize;</i> | Price <i>et al.</i> , 2002 ^{a,4} ; Ali <i>et al.</i> , 2014 ^{a,4} ; Brentner <i>et al.</i> , |

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| | | <i>Baccharis halimifolia; Populus deltoids, Popsolum notatum</i> | 2010 ^{b,3} |
| RDX | Growth inhibition | <i>Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maize</i> | Price <i>et al.</i> , 2002 ^{a,4} |
| RDX | Significant morphological impact to leaves and stems | <i>Brassica rapa, Cucumis sativa, Dactylis glomerata, Festuca arundinacea, Helianthus annuus, Lactuca sativa, Lespedeza capitata, Lolium perenne, Onobrychis viciifolia, Poa pratensis, Sanguisorba minor, Trifolium pretense, Trifolium repens, Triticum aestivum, Zea mays; Glycine max, Sorghum sudanese, Zea mays; Morella cerifera</i> | Winfield <i>et al.</i> , 2004 ^{a,1,2} ; Chen <i>et al.</i> , 2011 ^{a,b,2} ; Via <i>et al.</i> , 2014 ^{a,4} |
| RDX | Damage to photosynthetic functioning | <i>Morella cerifera</i> | Via <i>et al.</i> , 2014 ^{a,4} |
| RDX | Inhibitions germination | <i>Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays</i> | Khatisashvili <i>et al.</i> , 2009 ^{b,1,2} |
| RDX | Growth Promotion | <i>Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maize; Trifolium pretense, Triticum aestivum; Morella cerifera</i> | Price <i>et al.</i> , 2002 ^{a,4} ; Panz <i>et al.</i> , 2013 ^{a,2} ; Via <i>et al.</i> , 2015 ^{a,4} |
| Comp B | Damage to photosynthetic functioning | <i>Baccharis halimifolia; Morella cerifera</i> | Ali <i>et al.</i> , 2014 ^{a,4} ; Via <i>et al.</i> , 2014 ^{a,4} |
| Comp B | Synergistic impacts of RDX and TNT | <i>Triticum aestivu, Trifolium pratense</i> | Panz <i>et al.</i> , 2013 ^a |

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| Comp B | Antagonistic impacts of RDX and TNT | <i>Morella cerifera</i> | Via <i>et al.</i> , 2014 ^{a,4} |
| Comp B | Significant morphological impact to leaves and stems | <i>Baccharis halimifolia</i> ; <i>Morella cerifera</i> | Ali <i>et al.</i> , 2014 ^{a,4} ; Via <i>et al.</i> , 2014 ^{a,4} |

Table 1.2: Publications pertaining to the effects of explosives compounds at and across various life stages of vegetation. Observations are grouped based on the contaminant in question (i.e. RDX, TNT, and Composition B). Species tested are listed alphabetically and grouped by publication. Reference superscripts denote growing media used (^a = soil, ^b = hydroponics, ^c = agar).

| Contaminant | Life Stage | Species | Reference |
|-------------|-------------------|--|--|
| TNT | Germination | <i>Brassica rapa</i> , <i>Cucumis sativa</i> , <i>Dactylis glomerata</i> , <i>Festuca arundinacea</i> , <i>Helianthus annuus</i> , <i>Lactuca sativa</i> , <i>Lespedeza capitata</i> , <i>Lolium perenne</i> , <i>Onobrychis viciifolia</i> , <i>Poa pratensis</i> , <i>Sanguisorba minor</i> , <i>Trifolium pretense</i> , <i>Trifolium repens</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> ; <i>Lolium perenne</i> , <i>Medicago sativa</i> ; <i>Achillea millefolium</i> , <i>Achnatherum hymenoides</i> , <i>Agropyron Smithii</i> , <i>Amaranthus retroflexus</i> , <i>Andropogon Gerardii</i> , <i>Asclepias syriaca</i> , <i>Bouteloua gracilis</i> , <i>Datura stramonium</i> , <i>Elymus canadensis</i> , <i>Eragrostis trichoides</i> , <i>Ipomoea lacunose</i> , <i>Panicum virgatum</i> , <i>Polygonum pensylvanicum</i> , <i>Portulaca oleracea</i> , <i>Sida spinose</i> , <i>Sorghastrum nutans</i> ; <i>Gycine max</i> , <i>Oriza sativa</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> ; <i>Oriza sativa</i> ; <i>Cicer arietinum</i> , <i>Glycine max</i> , <i>Lathyrus sativum</i> , <i>Lolium multiflorum</i> , <i>Medicago sativa</i> , <i>Vigna sinensis</i> , <i>Vigna radiata</i> , <i>Zea mays</i> ; <i>Glycine max</i> , <i>Sorghum sudanese</i> , <i>Zea mays</i> ; <i>Morella cerifera</i> | Winfield <i>et al.</i> , 2004 ^a ; Best <i>et al.</i> , 2006 ^a ; Best <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2007 ^b ; Khatisashvili <i>et al.</i> , 2009 ^b ; Chen <i>et al.</i> , 2011 ^{ab} ; Via <i>et al.</i> , 2015 ^a |
| | Seedling/Juvenile | <i>Brassica rapa</i> , <i>Cucumis sativa</i> , <i>Dactylis glomerata</i> , <i>Festuca arundinacea</i> , <i>Helianthus annuus</i> , <i>Lactuca sativa</i> , <i>Lespedeza capitata</i> , <i>Lolium perenne</i> , <i>Onobrychis viciifolia</i> , <i>Poa pratensis</i> , <i>Sanguisorba minor</i> , <i>Trifolium pretense</i> , <i>Trifolium repens</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> ; <i>Gycine max</i> , <i>Oriza sativa</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> ; <i>Oriza sativa</i> ; <i>Cicer arietinum</i> , <i>Glycine max</i> , <i>Lathyrus sativum</i> , <i>Lolium multiflorum</i> , <i>Medicago sativa</i> , <i>Vigna sinensis</i> , <i>Vigna radiata</i> , <i>Zea mays</i> ; <i>Glycine max</i> , <i>Sorghum sudanese</i> , <i>Zea mays</i> ; <i>Morella cerifera</i> | Winfield <i>et al.</i> , 2004 ^a ; Vila <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2007 ^b ; Khatisashvili <i>et al.</i> , 2009 ^b ; Chen <i>et al.</i> , 2011 ^{ab} ; Via <i>et al.</i> , 2015 ^a |

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|-----|-------------------|--|---|
| | Adult | <i>Populus deltoids; Populus deltoids; Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maiz; Lolium perenne, Medicago sativa; Achillea millefolium, Achnatherum hymenoides, Agropyron Smithii, Amaranthus retroflexus, Andropogon Gerardii, Asclepias syriaca, Bouteloua gracilis, Datura stramonium, Elymus canadensis, Eragrostis trichoides, Ipomoea lacunose, Panicum virgatum, Polygonum pensylvanicum, Portulaca oleracea, Sida spinose, Sorghastrum nutans;</i> <i>Lolium perenne; Populus deltoids, Popsolum notatum; Populus deltoids, Panicum varigatum; Pinus albes, Pinus sylvestris; Morella cerifera; Pinus sylvestris, Picea glauca</i> | Thompson <i>et al.</i> , 1998a ^b ; Thompson <i>et al.</i> , 1998b ^b ; Price <i>et al.</i> , 2002 ^a ; Best <i>et al.</i> , 2006 ^a ; Best <i>et al.</i> , 2007 ^a ; Rocheleau <i>et al.</i> , 2008 ^a ; Brentner <i>et al.</i> , 2010 ^b ; Thompson and Polebitski 2010 ^a ; Schoenmuth <i>et al.</i> , 2014 ^a ; Via <i>et al.</i> , 2014; Schoenmuth <i>et al.</i> , 2015 ^a |
| | Across-Stages | <i>Gycine max, Oriza sativa, Triticum aestivum, Zea mays; Oriza sativa; Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays; Glycine max, Sorghum sudanese, Zea mays; Morella cerifera</i> | Vila <i>et al.</i> , 2007a ^a ; Vila <i>et al.</i> , 2007b ^a ; Khatisashvili <i>et al.</i> , 2009 ^b ; Chen <i>et al.</i> , 2011 ^{ab} ; Via <i>et al.</i> , 2015 ^a |
| RDX | Germination | <i>Festuca arundinacea; Bromus inermus, Festuca arundinacea; Lactuca sativa, Hordeum vulgare; Achillea millefolium, Achnatherum hymenoides, Agropyron Smithii, Amaranthus retroflexus, Andropogon Gerardii, Asclepias syriaca, Bouteloua gracilis, Datura stramonium, Elymus canadensis, Eragrostis trichoides, Ipomoea lacunose, Panicum virgatum, Polygonum pensylvanicum, Portulaca oleracea, Sida spinose, Sorghastrum nutans; Oriza sativa; Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays; Morella cerifera</i> | Peterson <i>et al.</i> , 1996 ^c ; Krishnan <i>et al.</i> , 2000 ^a ; Robidoux <i>et al.</i> , 2003 ^a ; Best <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2008 ^a ; Khatisashvili <i>et al.</i> , 2009 ^b ; Via <i>et al.</i> , 2015 |
| | Seedling/Juvenile | <i>Festuca arundinace; Bromus inermus, Festuca arundinacea; Lactuca sativa, Hordeum vulgare; Achillea millefolium, Achnatherum hymenoides, Agropyron Smithii, Amaranthus retroflexus, Andropogon</i> | Peterson <i>et al.</i> , 1996 ^c ; Krishnan <i>et al.</i> , 2000 ^a ; Robidoux <i>et al.</i> , 2003 ^a ; Kim <i>et</i> |

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| | | <i>Gerardii, Asclepias syriaca, Bouteloua gracilis, Datura stramonium, Elymus canadensis, Eragrostis trichoides, Ipomoea lacunose, Panicum virgatum, Polygonum pensylvanicum, Portulaca oleracea, Sida spinose, Sorghastrum nutans; Allium cepa; Oriza sativa; Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays; Morella cerifera</i> | <i>al.</i> , 2004 ^b ; Best <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2008 ^a ; Khatisashvili <i>et al.</i> , 2009 ^b ; Via <i>et al.</i> , 2015 |
| | Adult | <i>Cyperus esculentus; Brassica oleracea, Daucus carota, Lactuca sativa, Phaseolus vulgaris, Raphanus sativus, Valerianella locusta, Triticum aestivum; Populus deltoides; Acena sativa, Brassica rapa, Lepidium sativum, Triticum aestivum; Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maize; Cyperus esculentus, Lactuca sativa, Lycopersicon lycopersicum, Raphanus sativus, Zea maize; Baccharis halimifolia; Populus deltoids, Popsolum notatum; Morella cerifera; Morella cerifera</i> | Palazzo and Leggett, 1986 ^b ; Schneider <i>et al.</i> , 1996 ^a ; Scheidemann <i>et al.</i> , 1998 ^a ; Thompson <i>et al.</i> , 1999 ^b ; Gong <i>et al.</i> , 1999 ^a ; Price <i>et al.</i> , 2002 ^a ; Ait Ali <i>et al.</i> , 2006 ^a ; Brentner <i>et al.</i> , 2010 ^b ; Naumann <i>et al.</i> , 2010 ^a ; Via <i>et al.</i> , 2014 ^a |
| | Across-Stages | <i>Festuca arundinace; Bromus inermus, Festuca arundinace; Lactuca sativa, Hordeum vulgare; Achillea millefolium, Achnatherum hymenoides, Agropyron Smithii, Amaranthus retroflexus, Andropogon Gerardii, Asclepias syriaca, Bouteloua gracilis, Datura stramonium, Elymus canadensis, Eragrostis trichoides, Ipomoea lacunose, Panicum virgatum, Polygonum pensylvanicum, Portulaca oleracea, Sida spinose, Sorghastrum nutans; Oriza sativa; Cicer arietinum, Glycine max, Lathyrus sativum, Lolium multiflorum, Medicago sativa, Vigna sinensis, Vigna radiata, Zea mays; Morella cerifera</i> | Peterson <i>et al.</i> , 1996 ^c ; Krishnan <i>et al.</i> , 2000 ^a ; Robidoux <i>et al.</i> , 2003 ^a ; Best <i>et al.</i> , 2007 ^a ; Vila <i>et al.</i> , 2008 ^a ; Khatisashvili <i>et al.</i> , 2009 ^b ; Via <i>et al.</i> , 2015 |
| Comp B | Germination | <i>Trifolium pretense, Triticum aestivum</i> | Panz <i>et al.</i> , 2013 ^a |
| | Seedling/Juvenile | <i>Trifolium pretense, Triticum aestivum</i> | Panz <i>et al.</i> , 2013 ^a |
| | Adult | <i>Amaranthus retroflexus, Sorghastrum nutans; Baccharis halimifolia;</i> | Best <i>et al.</i> , 2009 ^a ; Ali <i>et al.</i> , |

| | | |
|---------------|--|---|
| | <i>Morella cerifera</i> | 2014 ^a ; Via <i>et al.</i> , 2014 ^a |
| Across-Stages | <i>Trifolium pretense</i> , <i>Triticum aestivum</i> | Panz <i>et al.</i> , 2013 ^a |

Figure Legends

Figure 1.1: Conceptual diagram showing linkages between the various facets of the explosives-vegetation interaction. The color of the gears represents the role it plays in relation to vegetation response to explosives. Red is source of contaminants, blue represents directly impacted factors, green natural processes, and orange indirect effects of the explosive compounds. The meters along the top of the figure show the interconnectedness of these concepts across both spatial and temporal scales. Design inspired by Walker and Wardle 2014.

Figure 1.2: Global map showing distribution of explosives. Country color represents particular levels of contamination (Clean/No Data, Low, Moderate, Medium, Heavy, and Very heavy contamination). Contamination data obtained from EPA, 2014; The Monitor, 2013; Japan Air Raids.org, 2015; THOR,2015.

Figure 1.3: Illustration of explosive compound uptake in vegetation. Diagram shows contaminants (RDX and TNT) leaching from munitions via contact with moisture and subsequently moving through soil in solution. This water is absorbed and transpired via evapotranspiration while the explosives, as shown by the red arrows, are sequestered by the plant.

Figure 1.4: Conceptual diagram of explosive compound interactions with vegetation across life stages and scale. Subset bullets show impacts of explosives at that particular life stage or spatial scale.

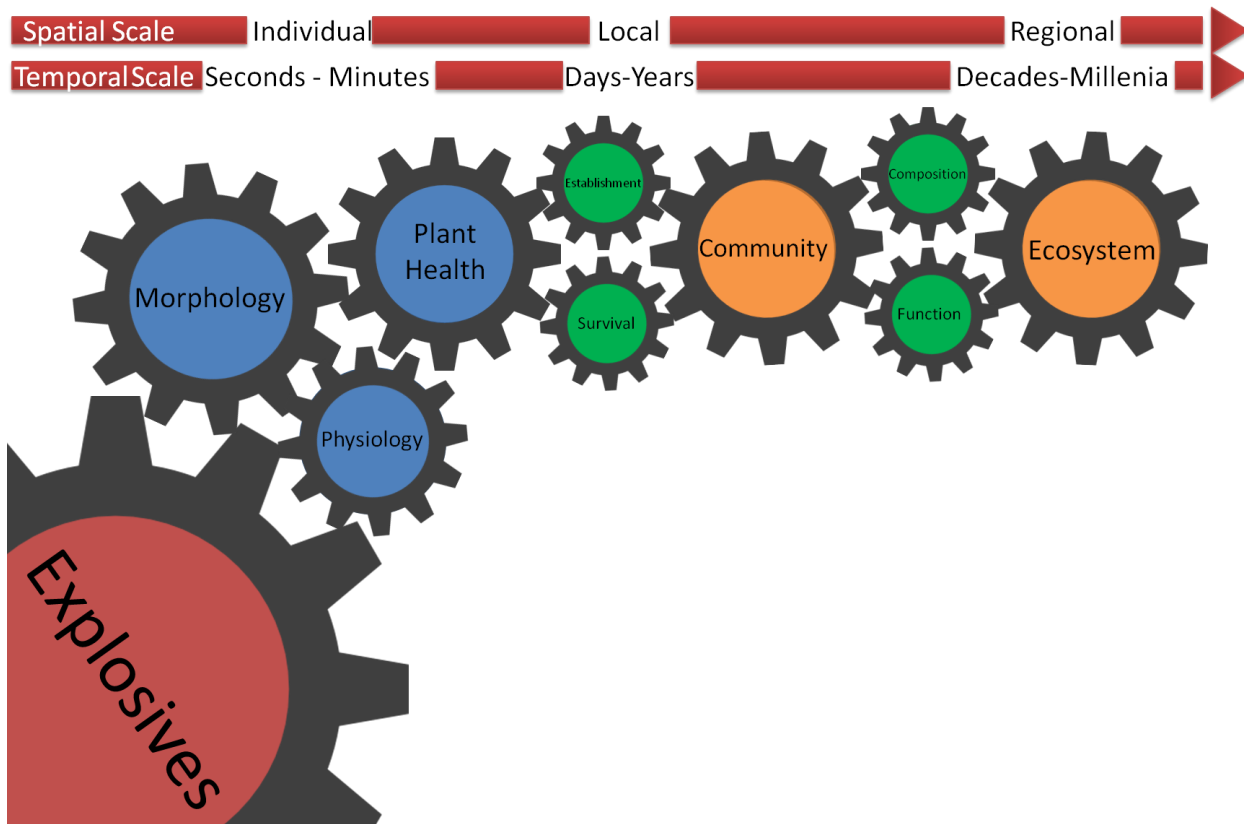


Figure 1.1

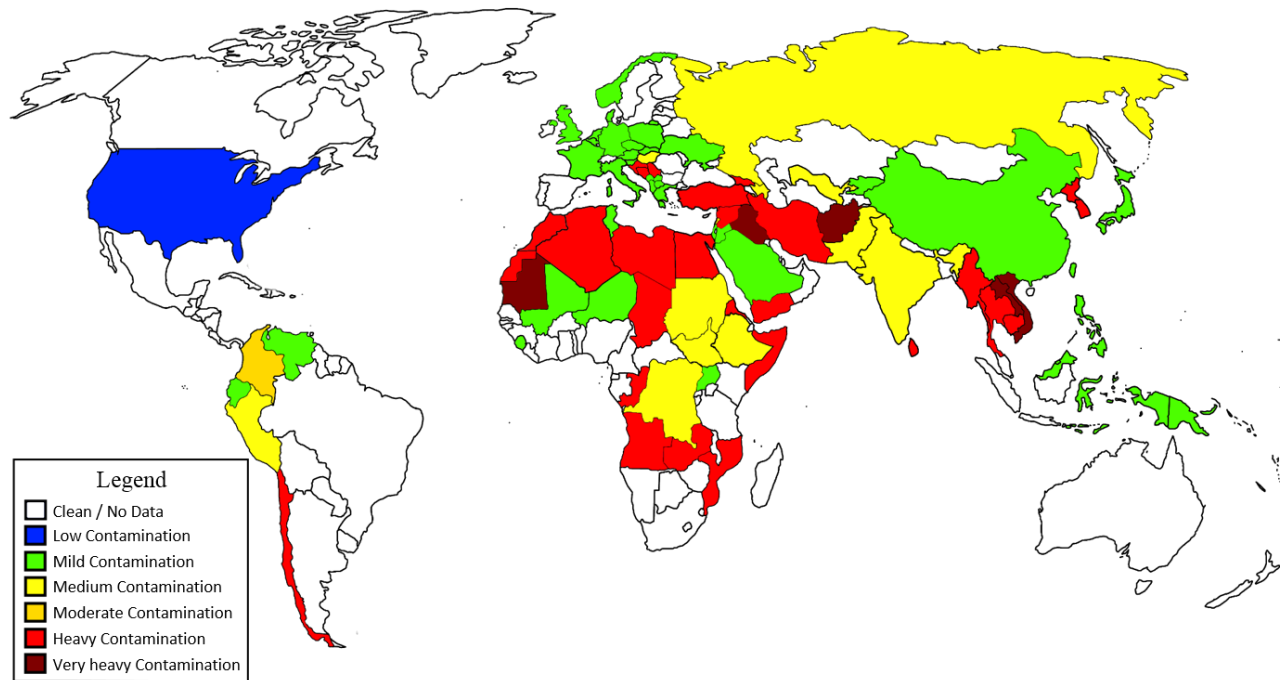


Figure 1.2

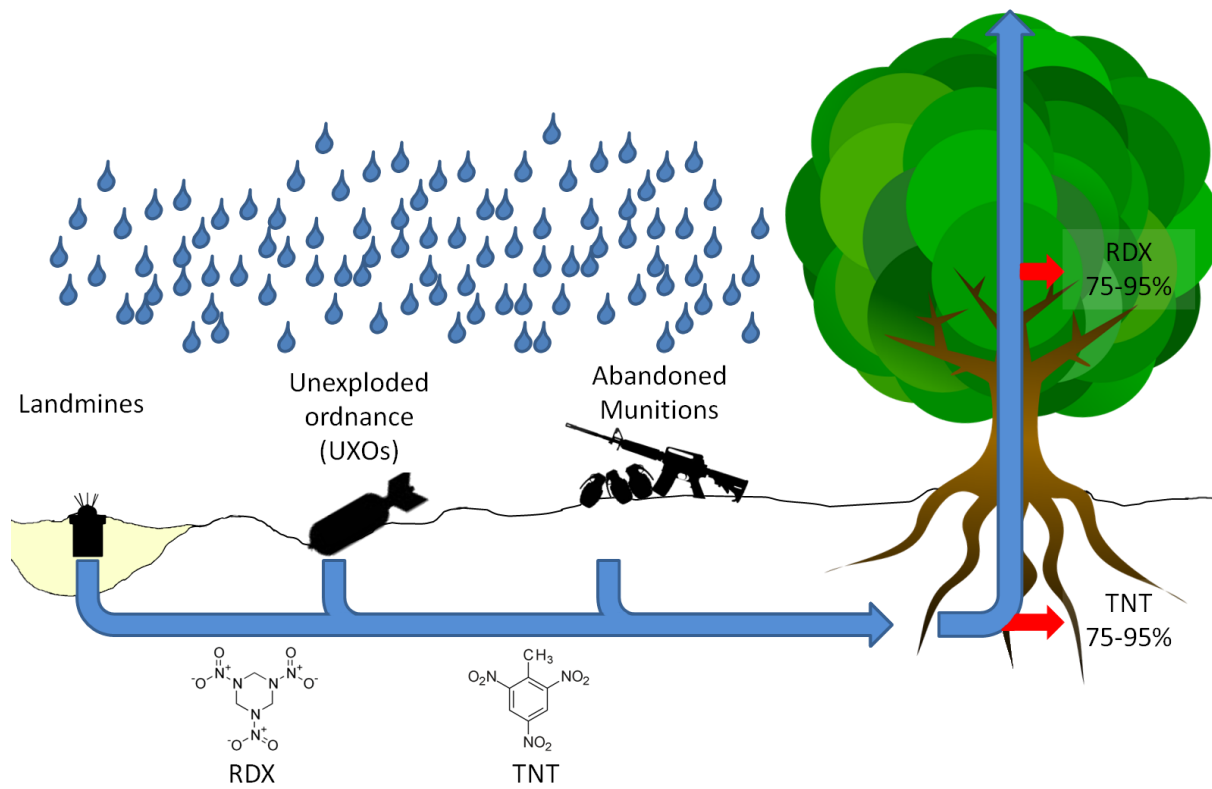


Figure 1.3

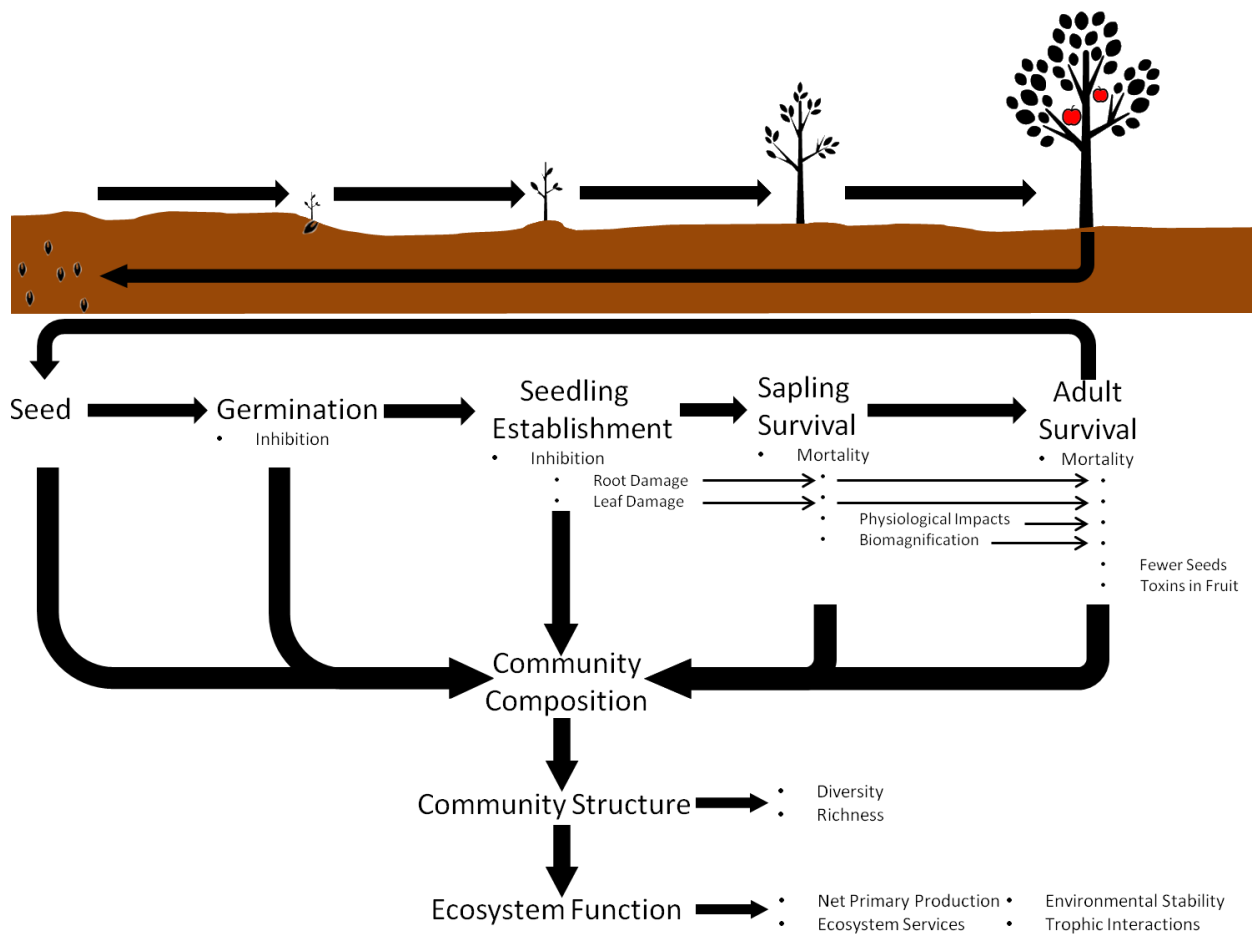


Figure 1.4

Chapter 2

Multiple metrics quantify and differentiate responses of vegetation to Composition B

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Introduction

Anthropogenic impacts on ecological systems have long been observed and documented. The magnitude of human influence has led to naming the current epoch the Anthropocene (Crutzen and Steffen, 2003). As part of human progress, many synthetic compounds have been developed and ended up in the environment (e.g.; xenobiotics; Sandermann, 1994). Explosive compounds are one such synthetic compound type which has seen heavy use. Among the number of explosive compounds created in the past, two compounds have seen a disproportionate amount of use, namely hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT). Due to prolific use, these two compounds are also the two most widely distributed organic explosive contaminants found in the environment (Hawari *et al.*, 2000; Rylott and Bruce, 2009; Khatisashvili *et al.*, 2009). In areas of military activity, the primary source of explosive compounds is from partially detonated and otherwise damaged munitions (Pichtel, 2012; Taylor *et al.*, 2015). The compounds these munitions contain have the potential to be highly mobile and as such leach out and readily interact with the nearby flora and fauna.

Morphological and physiological impacts, which tend to reflect localization of compounds in the plant, can be used to determine the influence of these compounds on vegetation (Via *et al.*, 2016). RDX is sequestered primarily in aerial tissues, impacting processes therein (Winfield *et al.*, 2004; Rodger, and D'Surney, 2004; Vila *et al.*, 2007; Via *et al.*, 2014), whereas TNT is sequestered predominantly belowground (Peterson *et al.*, 1998; Vila *et al.*, 2007; Khatsashvili *et al.*, 2009; Singh and Mishra, 2014). The most common morphological response to RDX is necrosis with other common responses including curled or irregular leaf margins, decreased leaf expansion, delayed emergence, and atypical bilateral symmetry (Winfield *et al.*, 2004; Rodger, and D'Surney, 2004; Vila *et al.*, 2007; Khatsashvili *et al.*, 2009; Singh and

Mishra, 2014; Via *et al.*, 2014). TNT damages existing root structures and hinders production of new roots (Peterson *et al.*, 1998; Gong *et al.*, 1999; Krishnan 2000; Vila *et al.*, 2007; Khatsashvili *et al.*, 2009; Singh and Mishra, 2014). Physiological responses to the presence of explosive compounds alter photosynthetic function as well as water relations (Thompson *et al.*, 1998; Ait Ali *et al.*, 2006; Zinnert, 2012; Zinnert *et al.*, 2012; Via *et al.*, 2014). Morphological damage can alter nitrogen and carbon content of leaves both in percent of the element present as well as the dominant isotope present (Dawson *et al.*, 2002). Physiological changes influence carbon assimilation through photosynthesis, carboxylation, gas exchange, and uptake of nitrogen which can alter the isotopic ratio of carbon in tissues (O'Leary, 1981; Dawson *et al.*, 2002). While morphology and physiology provide accurate instantaneous measurements, isotopes can provide a view into longer term effects on plant functioning (Dawson *et al.*, 2002). Physiological, morphological, and isotopic changes vary based on a number of factors including the contaminant in question (Via *et al.*, 2015), concentration of the contaminant (Pilon-Smits, 2005), species of vegetation involved (Scheidemann *et al.*, 1998), age of the individual (Inouye *et al.*, 2009; Via *et al.*, 2015), and condition of the surrounding area (Simini *et al.*, 2013; Kiiskila *et al.*, 2015).

Responses are further altered when multiple contaminants are present (Peralta-Videa *et al.*, 2002). Impacts of TNT and RDX in combination appear to have synergistic, additive, or conflicting impacts; the most common mixture being Composition B a 60:40 mixture of RDX and TNT respectively (Best *et al.*, 2007; Best *et al.*, 2009; Panz *et al.*, 2013; Ali *et al.*, 2014; Via *et al.*, 2014). Stress response similarities to RDX and TNT have been observed for vegetation with similar functional and life history traits (McIntyre *et al.*, 1995). For example, annuals are generally less impacted compared to perennials (Schnoor, *et al.*, 1995; Quist *et al.*, 2003; Zhang

and Chu, 2013), and monocots can be more tolerant than dicots (Winfield *et al.*, 2004; Rodger, and D’Surney, 2004; Vila *et al.*, 2007; Panz *et al.*, 2013). Although there are impacts to morphology and physiology which occur across species in the presence of explosives, there is high variation in degree of responses at the species level. With a thorough understanding of broad generalities in plant responses to explosive compounds, more accurate estimates of environmental effects can be formed. Quantifying impacts of explosives at a broader phylogenetic level can provide insight into the effects of explosives compounds at the ecosystem level. Our objective was to investigate impacts of the explosive mixture Comp B on plants representing various functional types and life history traits via morphology, physiology, and isotopic analyses. We hypothesized that multiple variables characterizing morphological and physiological responses will more clearly differentiate species and treatments than any one variable alone.

Methods

Plant Material

Plant species were chosen based on presence at sites contaminated with explosives along the Eastern United States. Adult plants of *Ulmus alata* (tree) and *Vitis labrusca* (liana) were ordered from Pinelands Nursery and Supply (Columbus, NJ), pruned, and allowed to grow through winter and spring (~6 months) in a glasshouse prior to experimentation. Seeds of *Cyperus esculentes* (graminoid/sedge, C₄ photosynthetic pathway) were ordered from the Dirty Gardener (www.amazon.com) and allowed to grow for a month, post-emergence, prior to experimentation. Species represent various physical and functional characteristics (functional type).

Soil contamination

Plants were grown in a 3:1 mixture of low nutrient (<5% nitrogen) topsoil and sand (Via *et al.*, 2014; Via *et al.*, 2015). This mixture approximated natural organic and nutrient content of field soils. The soil was amended with a solution of Composition B (provided by the Army Corps of Engineers) in 200 ml of acetone to bring the relative concentration to 500 mg Comp B kg⁻¹ soil (ppm; Ait Ali *et al.*, 2006; Naumann *et al.*, 2010; Zinnert *et al.*, 2012). Following treatment, the soil was kept at room temperature (~25° C) in the dark for 72 h to allow for the acetone to evaporate and prevent photodegradation of compounds (Ait Ali *et al.*, 2006). Reference plant soil was treated with 200 ml of uncontaminated acetone and was kept in the dark for 72 h prior to use.

Plant Physiology and Morphology

All response metrics were recorded during peak growing season for the region (July). Net photosynthesis (A_{NET} $\mu\text{mol m}^{-2}\text{s}^{-1}$) and stomatal conductance to water vapor diffusion (g_s $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) were measured at midday (1100-1300; 37°32'41.53"N) using an infrared gas analyzer (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE) portable infrared gas analyzer at 700 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of incident photosynthetically active radiation (PAR). Chlorophyll fluorescence measurements of PSII operating efficiency ($\Delta F/F_m'$) and maximum operating efficiency (F_v/F_m) were recorded using a pulse amplitude modulated fluorometer (Mini-PAM, Walz, Germany). Prior to measuring F_v/F_m plants were dark adapted for 30 min (Naumann *et al.*, 2010). Dark-adapted and light-adapted measurements of chlorophyll fluorescence were conducted on the third fully expanded leaf from the top of each plant. Maximum electron transport rate (ETR) was calculated as: $[\Delta F/F_m'] * \text{PAR}$, using 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ as the level of incident PAR. Midday leaf

water potentials (Ψ_{xylem}) were determined using a pressure chamber (model 1000, PMS Instrument Company, Albany, OR).

Total leaf count, necrosis, curling, and reduced leaf count were cumulatively measured for 8 weeks. Leaves were defined as necrotic if $\geq 30\%$ surface area was covered in lesions, as curled if the leaf deviated $\geq 45^\circ$ from the plane of the mid-vein, and as reduced if at maturation was $< 50\%$ size of mature reference leaves. Leaf area was recorded and separated into three categories: new (leaves formed after transplant), old (leave present prior to transplant) and total (new and old area combined). Biomass was harvested and weighed at the end of the experiment.

Isotopic Analysis

Elemental analyses included percent carbon (%C), percent nitrogen (%N), carbon to nitrogen ration (C:N), carbon 13 ($\delta^{13}\text{C}$), and nitrogen 15 ($\delta^{15}\text{N}$) from samples collected for root and leaf or above and below biomass ($n = 5$). Sample preparation included drying the leaves for 72 h, grinding leaves to a fine powder with a mortar and pestle, and passing it through a fine mesh (350 μm) sieve. Weighing and isotope analyses were conducted at the Cornell University Stable Isotope Laboratory (COIL), Ithaca NY, USA.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to identify significant ($\alpha=0.05$) variations in physiology and morphology across treatments and species as well as the interaction of these two factors. Where significance was found a one-way ANOVA was used to investigate differences between treatment and reference groups for each variable. All data were analyzed using JMP Pro 12 (©SAS Institute Inc.; Zar, 2010). Principal components analysis (PCA) was

used to show grouping of species based on morphological and physiological responses (PC-ORD software ver. 5.10).

Results

Individual Responses:

Significant impacts of explosives on morphology and physiology occurred across species, but observed responses varied (Table S1). Biomass was significantly reduced for aboveground tissues in *Vitis labrusca* (F=8.03, p=0.022) and *Cyperus esculentes* (F=15.53, p=0.004) and belowground tissues in *Ulmus alata* (F=6.10, p=0.039) and *C. esculentes* (F=10.78, p=0.011; Figure 2.1). No clear distinctions between species responses to exposure to Comp B were identified based on any one variable; however, trends were present for water relations and isotopic composition although not always significant. The only variables to respond consistently (but not significantly) across species were increases in g_s , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ (F=1.08 p=0.32, F=3.82 p=0.09, F=2.86 p=0.13 respectively; Table A1) and depletion of $\delta^{15}\text{N}$ (F=7.05 p=0.029, F=3.03 p=0.120, and F=28.18 p=0.0007 respectively; Figure 2.2)

Elemental and isotopic nitrogen and carbon changes were observed for all three species in the presence of explosive compounds (Table 2.1). Percent nitrogen in leaf tissues significantly decreased in the presence of explosives for *V. labrusca* (F=215.86, p<0.0001). Percent carbon of leaves was significantly reduced only for *C. esculentes* individuals in contaminated soils (F=8.76, p=0.018). Reduced C:N was observed in *U. alata* (F=9.18, p=0.016) yet increases were observed for *V. labrusca* (F=102.46, p<0.0001). Isotopic shifts in leaf tissues were also present for all three species. Significant declines in $\delta^{15}\text{N}$ were present in both *U. alata* (F=7.05, p=0.029) and *C. esculentes* (F=28.18, p=0.0007). Carbon 13 ($\delta^{13}\text{C}$) was enriched in both *U. alata*

($F=16.26$, $p=0.0038$) and *V. labrusca* ($F=43.81$, $p=0.0002$) individuals exposed to explosives but depleted in *C. esculentes* ($F=61.79$, $p<0.0001$). Root tissues showed similar shifts in carbon and nitrogen composition. Percent N was significantly reduced for *V. labrusca*, ($F=78.95$, $p<0.0001$) and %C was reduced for *C. esculentes* ($F=7.409$, $p=0.0262$). Changes in root C:N were observed in *V. labrusca* seen in leaves as well was still present ($F=148.29$, $p<0.0001$). Isotopic shifts for nitrogen in root tissues were present only for *V. labrusca* ($F=6.87$, $p=0.031$) which exhibited significant enrichment in $\delta^{15}\text{N}$. Enrichment of with $\delta^{13}\text{C}$ occurred in roots of *U. alata* ($F=9.80$, $p=0.0140$) and *V. labrusca* ($F=59.20$, $p<0.0001$) individuals exposed to explosives but depletions in *C. esculentes* ($F=84.68$, $p<0.0001$).

Combined Responses – all species:

When investigating morphological variables alone for all three species, the first two axes of the principal components analysis (PCA) explain 55% of the variation and clearly separated both species and treatment for *U. alata* and *V. labrusca* (Axis 1 = 31% and Axis 2= 24%; Figure 2.3a). The main variables for Axis 1 differentiation were old leaf area ($r=-0.87$), total leaf area ($r=-0.95$), and above ($r=-0.84$) and belowground biomass ($r=-0.85$). Separation along Axis 2 was driven solely by height ($r=-0.74$) and stem number ($r=-0.82$). The PCA of physiological responses showed a clear delineation of species as well as some separation of treatment groups across both axes which explained 64% of the variation (Axis 1 = 39% and Axis 2= 25%; Figure 2.3b). Separation along Axis 1 was driven by g_s $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ($r=-0.72$), Ψ_{xylem} ($r=0.76$), and A_{NET} $\mu\text{mol m}^{-2}\text{s}^{-1}$ ($r=0.80$) and Axis 2 by F_v/F_m ($r=-0.75$). Nitrogen and carbon isotopic data gave the greatest percent variance explained (71%; Axis 1 = 50% and Axis 2 = 21%) with all parameters influencing grouping (Figure 2.3c). Separation along axis 1 was due to %N ($r=-0.75$), and $\delta^{13}\text{C}$ ($r=0.84$) in leaves, as well as %N ($r=-0.76$) and $\delta^{13}\text{C}$ ($r=-0.91$) in roots. Axis 2 was

predominantly influenced by leaf $\delta^{15}\text{N}$ ($r=0.80$). Isotopes appeared to be the best set of variables for pulling *C. esculentes* individuals from the other two species as well as distinguishing reference and treatment values.

Combining morphological, physiological, and isotopic changes resulted in the best overall separation of both species and treatment groups (Figure 3d). Axes 1 and 2 explain 50% of the overall variation (Axis 1 = 32% and Axis 2 = 18%). Axis 1 separation was driven by old leaf area ($r=-0.84$), total leaf area ($r=-0.90$), above ($r=-0.90$), and belowground biomass ($r=-0.80$), g_s $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ($r=-0.71$), Ψ_{xylem} ($r=-0.77$), leaf %N ($r=-0.77$), leaf $\delta^{13}\text{C}$ ($r=0.78$), root % N ($r=-0.76$), and root $\delta^{13}\text{C}$ ($r=0.87$). Separation along Axis 2 was driven by height ($r=-0.83$), and stem number ($r=-0.83$).

Combined Responses – select species:

Because *C. esculentes* is a C_4 grass, we removed it from the PCAs to identify which factors were vital to the differentiation of *U. alata* and *V. labrusca* and between the reference and treatment values. . The morphology PCA showed separation for the treatment groups and species. The first two axes explained 59% of the variation (Axis 1 = 36% and Axis 2 = 23%; Figure 2.4a). Separation was driven by old leaf area ($r=0.73$), total leaf area ($r=0.85$), stem number ($r=0.75$), reduced leaf count ($r=0.74$), and belowground biomass ($r=-0.82$) for Axis 1 and by new leaf area ($r=-0.86$), and height ($r=0.74$) along Axis 2. Physiology alone produced a clear separation of reference and treatment with minor mixing (Figure 2.4b). The first two axes explained 70% of the variation (Axis 1 = 39% and Axis 2 = 31%). Separation observed along Axis 1 was caused by Ψ_{xylem} ($r=0.74$), $\Delta F/F_m'$ ($r=-0.76$), and A_{NET} $\mu\text{mol m}^{-2}\text{s}^{-1}$ ($r=0.81$) while that along Axis 2 was predominantly due to the influence of F_v/F_m ($r=0.84$). Isotopic data produced the clearest divide of all three variable types individually (Figure 4c) with axes 1 and 2

explaining 65% of the variation (Axis 1 = 43% and Axis 2 = 22%). Leaf %N ($r=0.80$), leaf $\delta^{15}\text{N}$ ($r=0.75$), root %N ($r=0.86$), and root $\delta^{13}\text{C}$ ($r=-0.90$) were the variables responsible for separation along Axis 1 and leaf $\delta^{13}\text{C}$ ($r=0.86$) and root %C ($r=-0.76$) were responsible for those along Axis 2.

Combining all variables for the woody species produced the clearest grouping for both species and treatment. The first two axes explained 53% of the variation (Axis 1 = 31% and Axis 2 = 22%; Figure 2.4d). Separation along Axis 1 was due to influences of old leaf area ($r=0.76$), total leaf area ($r=0.80$), aboveground biomass ($r=0.71$), Ψ_{xylem} ($r=-0.78$), leaf $\delta^{15}\text{N}$ ($r=0.77$), root %N ($r=0.73$), and root $\delta^{13}\text{C}$ ($r=-0.71$). Separation along Axis 2 was driven by new leaf area ($r=-0.79$), height ($r=0.82$), F_o ($r=-0.74$), and leaf $\delta^{13}\text{C}$ ($r=-0.80$).

Discussion

Our goal was to distinguish the impacts of Comp B on vegetation using a number of common metrics. Our hypothesis was supported; multiple variables representing morphological and physiological responses clearly differentiated species and treatments better than any one variable alone. Morphological and physiological responses to Comp B for woody species (*Ulmus alata* and *Vitis labrusca*) were more similar as compared to the herbaceous species, *Cyperus esculentes*. Other studies have reported differences in response to explosives based on species and functional group (Via and Zinnert, 2016) and on individual variables. In our study no one variable definitively separated out treatment from reference groups.

Variations in $g_s \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ cannot be explained by any other measured morphological or physiological variable. Given that Comp B is 40% TNT which is more phytotoxic than RDX and largely influences belowground structures (Peterson *et al.*, 1996; 1998;

Gong *et al.*, 1999; Pilon-Smits, 2005; Vila *et al.*, 2005; Zinnert, 2012; Via *et al.*, 2014), we could expect significant reductions in g_s $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$. In our study, Comp B elicited increased conductance values, a trend that is also observed for RDX exposure (Via *et al.*, 2014). Since RDX is predominantly stored in aboveground tissues (Price *et al.*, 2002; Ali *et al.*, 2014; Brentner *et al.*, 2010) and transported throughout the plant body via the transpiration stream (Yoon *et al.*, 2005; Brentner *et al.*, 2010). Variations in gas exchange observed could be due to impacts on leaf tissues or possibly some direct impact to the hydraulic processes. This is particularly interesting given the high variability of morphological and physiological responses between the species. Water related processes may be altered when explosives are present in the soil (Thompson *et al.*, 1998; Kim *et al.*, 2004; Naumann *et al.*, 2010; Via *et al.*, 2014) suggesting that water relations may be an important focus in future studies.

Nitrogen and isotopic analyses provided a unique insight into species response to the presence of Comp B as it integrates information about morphological condition and physiological function (Dawson *et al.*, 2002). Nitrogen isotopic composition responded similarly across species when exposed to Comp B. Depletions of $\delta^{15}\text{N}$ were observed for all three species in the presence of Comp B suggesting that each had greater access to the lighter ^{14}N than reference individuals. The primary source of ^{14}N available to individuals was from the Comp B present in the soil, suggesting that the explosive compounds was a nitrogen source (Khatisashvili *et al.*, 2009). Explosive compounds are depleted in ^{15}N due to the manufacture process, although this can vary among manufacturers (Howa *et al.*, 2014). Through degradation processes due to endophyte and soil microbial activity (Burken and Schnoor, 1997; Hannink *et al.*, 2002; Wenzel, 2009; Strand *et al.*, 2014), it is possible that the nitrogen from the explosive compounds is available to the plants (Bernstein and Ronen, 2012), or possibly through some degradative or

transformative action of the plant itself (Sandermann, 1994; Just and Schnoor, 2004; Yoon *et al.*, 2005). Previous studies have shown biomass increases in the presence of explosives lending support to this idea (Gong *et al.*, 1999; Khatisashvili *et al.*, 2009).

Despite the limited number of responses with trends that span all species tested, there were several generalities which surfaced through multivariate analysis. Common traits which were vital to differentiating the different groups included measures of growth (e.g. leaf area and stem number), hydraulic characteristics, and nitrogen composition for both leaves and roots. As we hypothesized, comparing multiple variables produced clearer delineation of both treatment groups and species. Morphological variables provided clear separation and were effective in separating exposed and reference groups regardless of species. This is particularly promising as morphological metrics have been used as the standard suite of characteristics to observe plant responses to explosives (Palazzo and Leggett, 1986; Peterson *et al.*, 1998; Krishnan *et al.*, 2000; Gong *et al.*, 1999; Price *et al.*, 2002; Winfield *et al.*, 2004; Rodger, and D'Surney, 2004; Ali *et al.*, 2006; Khatisashvili *et al.*, 2009; Chen *et al.*, 2011; Via *et al.*, 2014). Common metrics for aboveground impacts focus on leaf damage (i.e. necrosis, chlorosis, curling, leaf margin damage) were not significant in differentiating species or treatments in our study, yet other morphological characteristics, particularly those associates with growth, were vital in the separation of various groups (i.e. height, leaf area, biomass).

Physiological characteristics, driven predominantly by hydraulic related processes provided greater separation of species and treatment groups. Hydrology is the primary avenue through which explosives migrate into and throughout the plant body due to the structure and behavior of the compounds (Yoon *et al.*, 2005; Singh and Mishra, 2014). Hydraulic function has also been observed to respond, both positively and negatively, to the presence of explosives

across several species (Thompson *et al.*, 1998; Ali *et al.*, 2006; Zinnert, 2012; Zinnert *et al.*, 2012; Via *et al.*, 2014).

Nutrient and isotopic data provided the most distinct separation of *U. alata*, *V. labrusca*, and *C. esculentes*. Isotopes are an integrative metric of both morphological and physiological conditions that give insight into long term changes within the plant. Nitrogen and carbon composition do not change rapidly, as many other more commonly used metrics do (Dawson *et al.*, 2002). Among all variables that we tested, isotopic data were the most consistent in response. Although it will not replace high performance liquid chromatography (HPLC) as a detector of compound uptake, it may be a useful addition in understanding variable plant responses to explosives exposure (including species ideal for phytoremediation). Variations in responses after exposure to a stressor span both morphology and physiology, and not all are visibly detectable (Chapin III, 1991). The only isotopic parameter that was not influential in driving group separation was %C. Leaf and root nitrogen and carbon isotopic composition were particularly influential even when all other variables were included. Identifying commonalities across broad vegetation functional groups in responses elicited from exposure to explosive compounds enable more rapid estimation of field impacts and simplifies remediation strategies. Some established response generalities can be made for several functional groups. For instance; annuals are less susceptible to disturbance and contamination than perennials (Schnoor, *et al.*, 1995; Quist *et al.*, 2003; Zhang; Chu, 2013) and monocots are generally more tolerant of explosives than dicots (Winfield *et al.*, 2004; Rodger and D'Surney, 2004; Panz *et al.*, 2013).

Examining various morphological and physiological characteristics for vegetation exposed to explosives allows for differentiation of both species and stress response. Combining multiple variables allows for greater accuracy than any one variable alone. The literature is

largely dominated by studies of morphological impacts to vegetation and plant sequestration/degradation capabilities (Via and Zinnert, 2016). We show that morphology may be used to differentiate treatment and reference individuals; however, it was not the most effective approach. The inclusion of various metrics can greatly increase the ability to identify and differentiate particular groups. By using multivariate analyses and standard vegetation metrics, new aspects of the vegetation response to explosive compounds may be identified. Novel metrics can reinforce previously established metrics and concepts as well as provide a new perspective. Through $\delta^{15}\text{N}$ for instance, it can be inferred that there is uptake and assimilation of nitrogen from the explosives, as has been shown previously (Scheidemann *et al.*, 1998; Price *et al.*, 2002; Brentner *et al.*, 2010; Ali *et al.*, 2014), and via $\delta^{13}\text{C}$ changes to water relations and photosynthesis can also be inferred supporting our results and those published in previous works (Ali *et al.*, 2006; Naumann *et al.*, 2010; Via *et al.*, 2014). Gaining a more complete understanding of the explosive plant interaction, particularly for fine scale processes, opens new avenues of study and application. Tracing the pattern of impacts in vegetation can improve understanding of explosive compound behavior aiding in compound tracking and field prediction of impacts.

Table 2.1: Mean (\pm standard error) nitrogen and carbon values for roots and leaves of all three species.

| | <i>Ulmus alata</i> | | <i>Vitis labrusca</i> | | <i>Cyperus esculentes</i> | |
|------|--------------------|------------------|-----------------------|------------------|---------------------------|------------------|
| Leaf | Reference | Comp B | Reference | Comp B | Reference | Comp B |
| %N | 0.95 \pm 0.06 | 1.02 \pm 0.11 | 1.55 \pm 0.04 | 0.87 \pm 0.01 | 0.71 \pm 0.05 | 0.65 \pm 0.07 |
| %C | 46.25 \pm 0.49 | 39.86 \pm 4.48 | 47.68 \pm 0.37 | 44.56 \pm 1.67 | 40.67 \pm 0.95 | 30.08 \pm 3.45 |
| C:N | 49.31 \pm 3.15 | 38.95 \pm 1.35 | 30.93 \pm 0.96 | 50.95 \pm 1.73 | 58.84 \pm 5.29 | 46.24 \pm 1.41 |
| Root | | | | | | |
| %N | 1.89 \pm 0.39 | 1.39 \pm 0.04 | 2.76 \pm 0.15 | 1.19 \pm 0.09 | 1.15 \pm 0.12 | 0.98 \pm 0.06 |
| %C | 45.56 \pm 0.79 | 46.56 \pm 1.86 | 44.77 \pm 0.57 | 42.79 \pm 2.24 | 43.13 \pm 0.38 | 39.40 \pm 1.32 |
| C:N | 27.92 \pm 4.81 | 33.47 \pm 1.22 | 16.38 \pm 0.77 | 36.34 \pm 1.45 | 39.04 \pm 3.87 | 40.75 \pm 1.86 |

Figure Legends

Figure 2.1: Aboveground and belowground biomass across the three species. Reference values are in black and Composition B treatment in grey. Significant differences between reference and treatment values are denoted with an asterisk (*).

Figure 2.2: Leaf isotope values for both $\delta^{12}\text{N}$ and $\delta^{13}\text{C}$. Reference values are in black and Composition B treatment in grey. Significant differences between reference and treatment values are denoted with an asterisk (*).

Figure 2.3: Principal components analysis ordinations of tested variables from all three species separated into four groups: A) morphology, B) physiology, C) isotopic values, and D) all measurements combined. Solid shapes represent reference values while hollow ones represent Composition B treatment values. Dotted lines denote separation of groups. Correlations >0.7 are listed alongside each axis. Location does not denote direction or magnitude of influence.

Figure 2.4: Principal components analysis ordinations of tested variables from both woody species (*U. alata* and *V. labrusca*) separated into four groups: A) morphology, B) physiology, C) isotopic values, and D) all measurements combined. Solid shapes represent reference values while hollow ones represent Composition B treatment values. Dotted lines denote separation of groups. Correlations >0.7 are listed alongside each axis. Location does not denote direction or magnitude of influence.

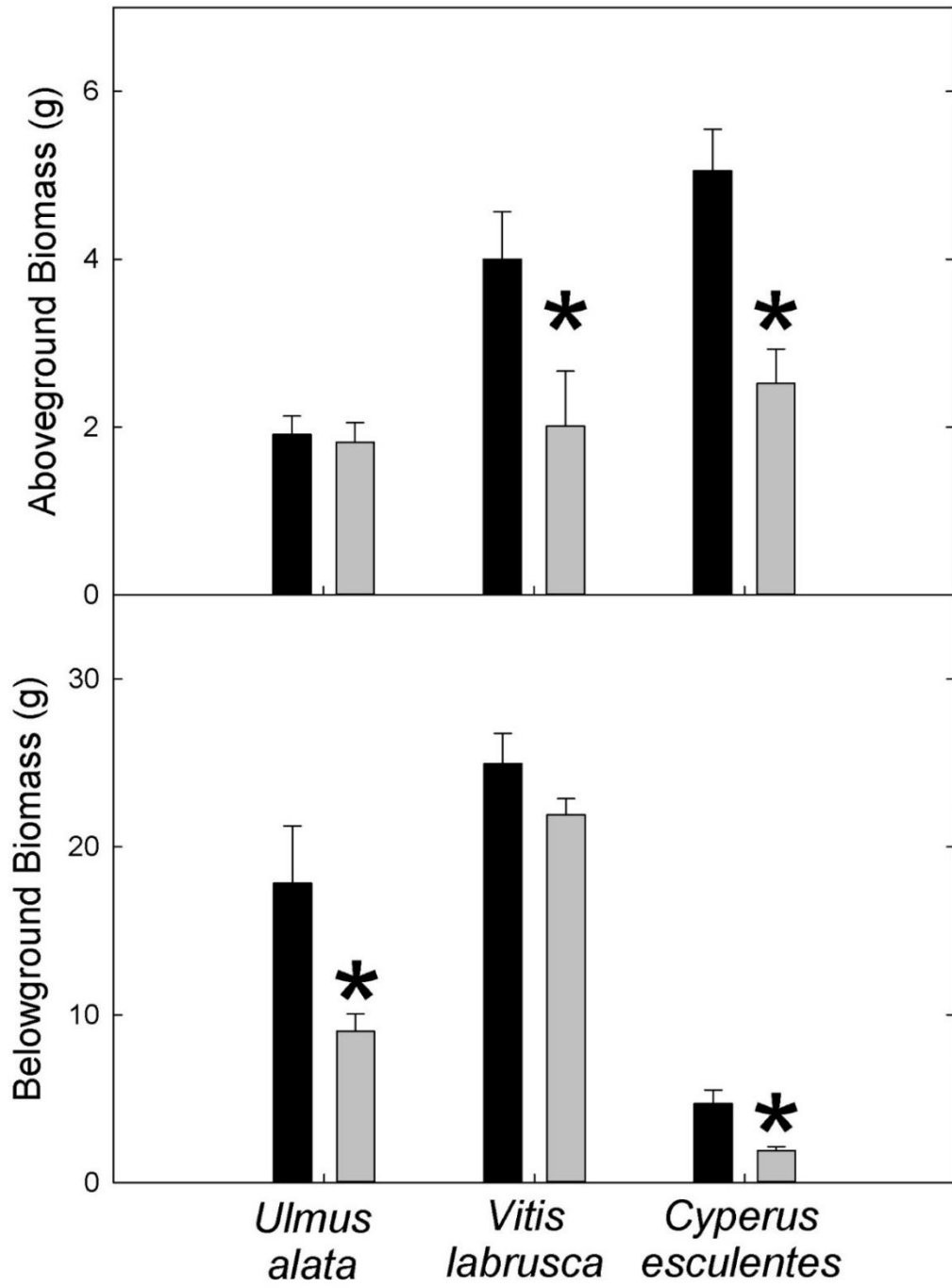


Figure 2.1

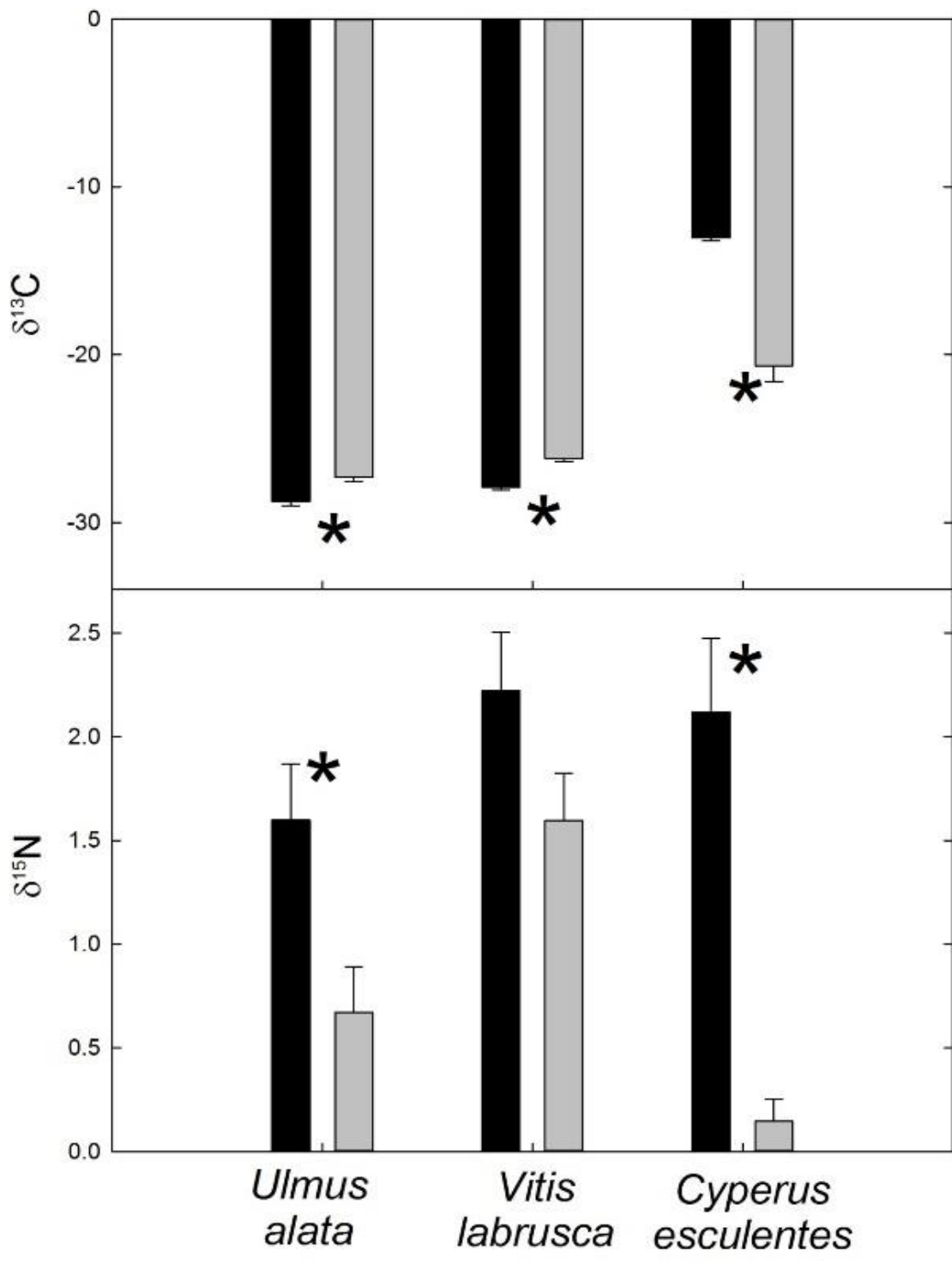


Figure 2.2

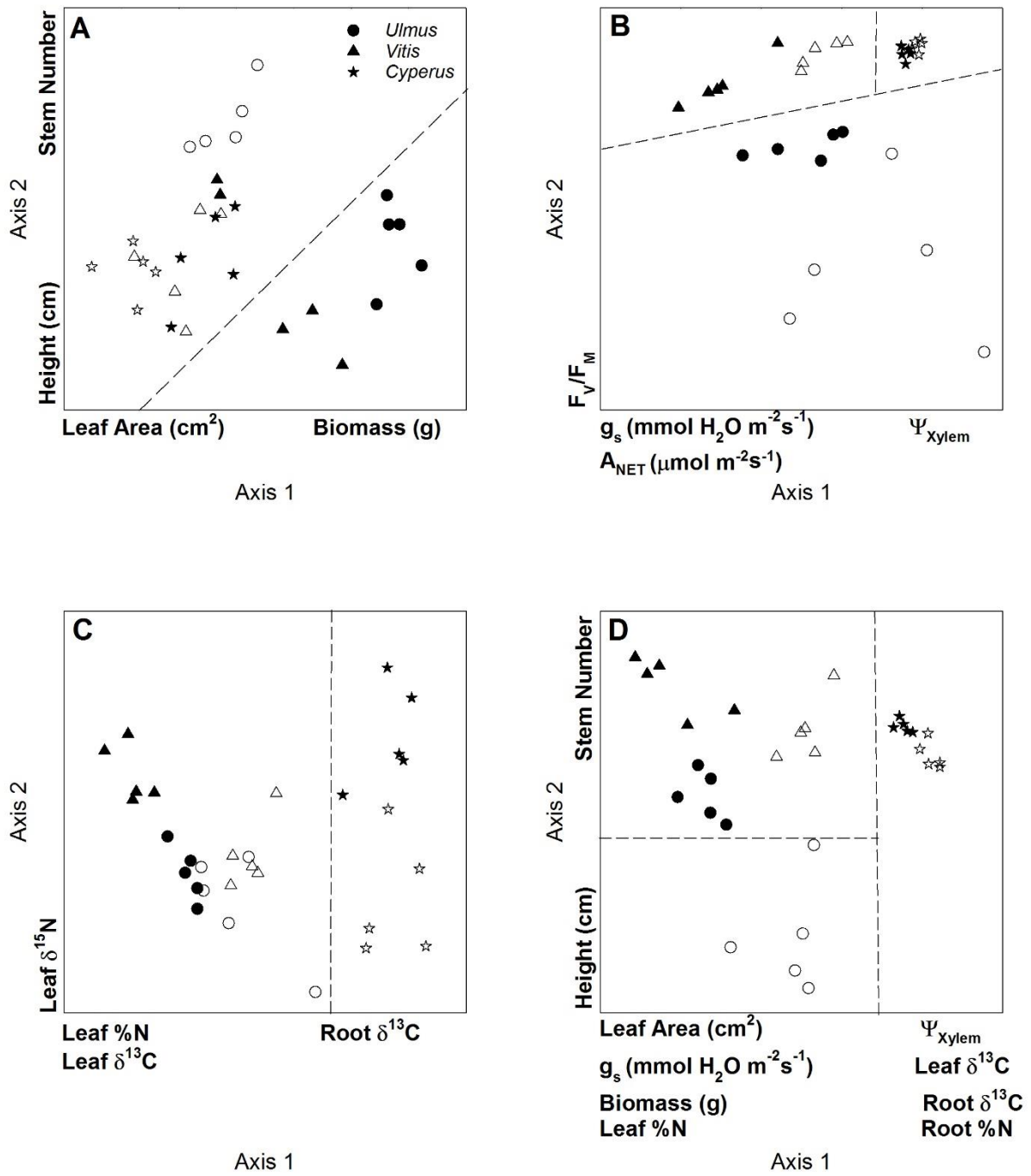


Figure 2.3

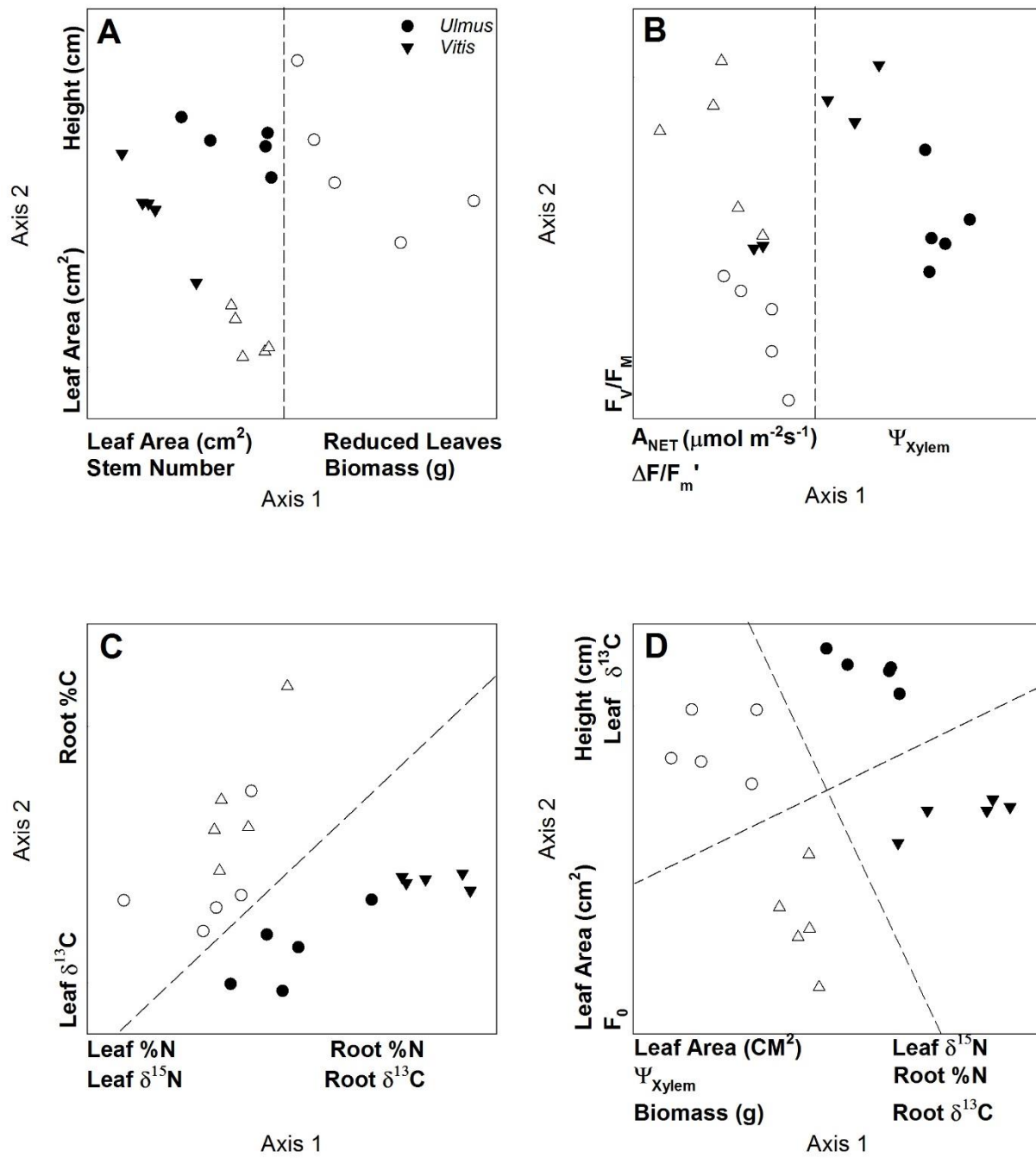


Figure 2.4

Chapter 3

Legacy Effects of Explosive Contamination on Vegetative Communities

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Introduction

Natural selection pressures act as filters for establishment and succession of vegetative species. Via dispersal, environmental, and physiological filters, community composition is steered to best benefit the condition of any given area (Lambers *et al.*, 2008; Hanson *et al.*, 2016). Presence of anthropogenic contaminants add an additional filter to the system, reducing fecundity of sensitive species, and increasing that of tolerant ones (Via and Zinnert, 2016). Loss or suppression of dominant/key species can have significant effects on ecosystem function (Newman and Clements, 2007) but even changes in non-dominant species density can have significant community level impacts (Kim *et al.*, 2015). Community shifts have been previously investigated and documented for a range of anthropogenic contaminants (Prach, *et al.*, 2014; Woch *et al.*, 2016) from mining operations (Brady and Noske, 2010; Zhang and Chu, 2011; Pandey *et al.*, 2014) to radiation exposure (Woodwell and Rebeck, 1967). Richness and diversity of plant species and functional diversity may be vital to ecosystem stability and function (Cardinale *et al.*, 2006; Cadotte *et al.*, 2009; Flynn *et al.*, 2011; Feng *et al.*, 2014).

Long term impacts of contaminants on vegetation are less studied than shorter exposure times (Via and Zinnert, 2016). This poses a problem as many anthropogenic contaminants are recalcitrant (i.e. heavy metals, explosives, agrochemicals, etc...; Klein and Scheunert, 1982; Rylott and Bruce, 2009; Russell, 2011) when released into the environment and eliciting chronic stress for many years in surrounding flora and fauna (Ansari *et al.*, 2015). Even short term exposure to anthropogenic contaminants may have long-lasting implications (Eschtruth and Battles, 2014). The persistent nature of these compounds adds to this complexity of community level responses. Chronic exposure over extended periods of time has significant impacts on

established communities (Holl, 2002; Newman and Clements, 2007; Travis *et al.*, 2008) and the seedbank of contaminated areas (Huopalainen *et al.*, 2000; Huopalainen *et al.*, 2001).

Once released into the environment anthropogenic contaminants exhibit a range of behaviors (Ansari *et al.*, 2015; Walsh *et al.*, 2015). Some rapidly bind to soil particles of organic matter, while others are not constrained to a static location (Cunningham *et al.*, 1995).

Compound mobility and structure play large roles in contaminant behavior in the environment (Pilon-Smits, 2005). For example, heavy metals behave differently from pharmaceuticals, agrochemicals, and explosives (Pilon-Smits and Freeman, 2006; Wemzel *et al.*, 2009).

Nanoparticles of a particular element behave very differently than larger particles of the same element (Zhuang *et al.*, 2015). As varied as the behavior of contaminants in the environment are there is equal variability in responses of vegetation to contaminants.

Impacts on vegetation can range from morphological damage to physiological interruptions and exact response depends on the contaminant and plant species in the target area. Plant functional traits (e.g. life history, leaf morphology, root structure, photosynthetic pathway, etc.) can also influence response to stress (Flynn *et al.*, 2009; Vandewalle *et al.*, 2014; Kusumoto *et al.*, 2015) and ability to persist in contaminated conditions. As plants age their biochemical processes change (Bond, 2000; Donaldson *et al.*, 2006; Juvany *et al.*, 2013). It may be from these alterations in biochemistry that various impacts to life stage occur. Mature plants have more biomass relative to earlier life stage which may allow the plant to withstand stress associated with the explosive compounds and then recover (Collins *et al.*, 2006). There are dualities present with tolerance to contaminants; plants capable of surviving may be harmed more as exposure time increases or may pass the contaminant through the system (Verkleij *et al.*, 2009). High

variability in impacts on vegetation based on species, functional group, and even life stage adds a selection pressure to contaminated areas.

Our study objective was to investigate long term impacts of explosives presence on the current vegetative community of a minefield contaminated 17 years prior with three different explosive compounds/mixtures. We hypothesized that 1) community richness and diversity in contaminated areas would not exhibit change from reference plots as species fill in vacant niches, 2) species composition would differ from reference plots as presence of contaminants filter out intolerant species, and 3) that changes in species functional traits would differ in contaminated treatment plots from reference.

Methods

Contaminants

Explosive compounds were chosen for this study as there is little vegetative community data from long standing field sites and have a broad global presence. The two most commonly used explosive compounds are hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2-methyl-1,3,5-trinitrobenzene (TNT). These compounds are the two most widely distributed organic explosive contaminants found in the environment (Hawari *et al.*, 2000; Rylott and Bruce, 2009; Khatisashvili *et al.*, 2009); both can have significant impacts on vegetation health. Munitions contain either a single compound (TNT most commonly) or, more commonly, a mixture of compounds (TNT and RDX). Composition B (Comp B) a mixture of RDX (60%) and TNT (40%) is a historically common munitions mixture. It was used extensively throughout World War II up through the 1950s, and is still used today (Pichtel, 2012).

Study site

Data were collected from a privately owned experimental minefield in South Carolina, USA (ORNL, 1999; Fischer *et al.*, 2000; Figure 3.1). Geologically the site is located on the Carolina terrane (ct) composed of clastic rocks in the upper region and intermediate to felsic pyroclastic rocks (>3 km) in the lower. The soil at the plots was Herndon very fine sandy loam and Kirksey silt loam, both with 2-6% slopes (USGS).

The property was owned and managed by Force Protection Inc. at the time of data acquisition. Initially used for a microbial study in 1999 this site was razed to the ground and sectioned off into 5x5 m plots. Within a number of those plots explosive compounds were buried in “dummy landmines” to mimic leaching of explosives from unexploded ordnance (UXOs). These dummy mines took the form of boxes filled with TNT (2-methyl-1,3,5-trinitrobenzene) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), or a mixture of the two compounds, Composition B (Comp B). Comp B is comprised of 60% RDX, 39% TNT, and 1% wax binder. Dummy devices were designed to be similar to antipersonnel landmines and are assumed to have contained between 75-125g of the designated explosive compound (Oxley *et al.*, 2003; Cardona *et al.*, 2014). Reference areas were established upslope of the contaminated plots in the razed area. Once the dummy devices had been planted the area was left to naturally revegetate.

In each of the treatment and reference areas 1x1m plots were delineated (Figure 1) and contaminated plots were checked using site maps from the property owner prior to data collection to ensure presence of a dummy device. Herbaceous species were identified and percent cover established in each plot. Woody species abundance was also collected after the species had been identified. Minimally destructive metrics were used due to site protocol.

Species Diversity

Species density and frequency were calculated for all species. Species richness (S), Shannon-Weiner (H'), and Simpson (D_1) metric values were calculated for total species as well as herbaceous and woody species separately. Both H' and D_1 were used to take into consideration both rare, which H' is sensitive to, and common species, which are emphasized by D_1 (McCune and Grace, 2002; Morris *et al.*, 2014). All species metrics were compared across treatment groups via analysis of variance (ANOVA) using a Tukey's *post-hoc* test.

Species Composition

Jaccard's index of similarity was calculated for total, herbaceous, and woody species composition to investigate whether similarities in species composition were present across groups. Cluster analysis (with Sorensen distance) was used to determine how the plots relate to one another based on species composition. To determine if the resulting groups were significantly different, a multi-response permutation procedure (MRPP) was used (with Sorensen distance). MRPP was followed by pairwise comparison to compare species composition among groups.

Functional Trait Composition

Due to constraints on site access, functional trait data for all species recorded in the field were aggregated from literature sources (Uva *et al.*, 1997; Porcher and Rayner, 2001; Bryson and DeFelice, 2009; Tredici, 2010; USDA, 2014). Functional traits included: life history, native status, growth form, habit, maximum height, root structure, leaf morphology, seed size, dispersal mechanism, and photosynthetic pathway. To standardize trait values, community weighted mean (CWM) for each plot were calculated using the FD package in R (Garnier *et al.*, 2004; Laliberté *et al.*, 2014). CWM allows categorical and continuous functional traits to be weighted by

abundance of representative species, limiting any bias which may come from rare species or traits. This allows for the identification of dominant traits and provides insight into ecosystem processes (Ricotta and Moretti, 2011). CWM values for each functional trait were compared across treatment groups via ANOVA and Tukey's *post-hoc* test. Values were also used in a cluster analysis and MRPP (both with Sorensen distance) to investigate plot grouping. MRPP was followed by a pairwise comparison to compare species composition among groups.

Results

Species Diversity

Woody species density was variable across treatment groups for most species with Composition B plots distinguishing themselves from both reference and other treatment plots (Figure 3.2). RDX and TNT showed no significant change in woody species density compared to reference plots. Comp B plots exhibited significantly higher density for *Ulmus alata* ($F=6.40$, $p=0.0016$) and *Quercus virginiana* ($F=3.92$, $p=0.017$) relative to reference values ($p=0.0002$ and $p=0.0067$, respectively). *Ligustrum sinense*, an invasive species, was common across all sites ($F=2.15$, $p=0.113$) but was significantly more dense in Comp B treated areas when compared to densities in TNT plots ($p=0.0211$). *Diospyros virginiana* was the only woody species which exhibited a significantly reduced presence in all three contaminated areas relative to reference (Figure 1; $F=6.11$, $p=0.0021$). Density of the other three woody species were similar across treatments; however, only one of those, *Rhus copallina*, was present in abundance (Figure 3.2).

Herbaceous species exhibited high variability in frequency and cover. Few species dominated the plots with a majority of the species recorded being rare (Appendix 1). *Oenanthera biennis* was the most prolific herbaceous species recorded with a dominant presence (>50%

percent cover) across treatment groups. *Rubus fruticosus*, *Panicum capillare*, *Campsis radicans*, *Dicanthylom sp.*, and *Lespedeza cuneate* were all dominant in at least one treatment area, and generally present in some quantity across all treatments.

No differences were observed in richness, diversity, or Simpson index for herbaceous and woody species combined ($p > 0.05$; Table 3.1). Separating out herbaceous and woody species did provide some differentiation of treatment groups. Species richness remained unchanged for herbaceous species yet woody species showed significant variation ($F = 3.37$, $p = 0.0314$), between Composition B and both RDX and TNT. Shannon-Weiner diversity, which is sensitive to rare species, was significantly different for herbaceous ($F = 4.41$, $p = 0.0105$) and woody ($F = 3.79$, $p = 0.0204$) plant communities (Table 3.1). Only woody species showed a significant difference between the reference and treatment groups. Simpson's index, which is sensitive to abundant species, also showed significant differences between treatment groups in herbaceous ($F = 5.32$, $p = 0.0043$) and woody ($F = 3.55$, $p = 0.0261$) species (Table 3.1). This metric was the only one to show a significant difference between the reference and a treatment group for both vegetation types. Overall it appeared that abundant species, rather than rare ones, were better able to differentiate reference and treatment communities suggesting shifts in species dominants with presence of explosives contamination.

Species Composition

Jaccard's index of similarity revealed that community composition across treatments was between 40 and 60% similar to one another (Table 3.2). Herbaceous community had similar values. The woody assemblage exhibited the least amount of similarity among treatments groups ranging from 14 to 43%. The reference in particular saw a large reduction for woody species exhibiting only 14-28% similarity.

Cluster analysis of species composition across treatments delineated three very distinct groups: 1) reference plots, 2) single contaminant plots (RDX and TNT), and 3) mixed contaminant plots (Composition B; Figure 3.3). Dendrogram grouping was supported by multi-response permutation procedure (MRPP) of species composition. MRPP showed that communities present in the plots were able to define the preexisting grouping scheme ($A=0.11$, $p<0.0001$). Pairwise comparisons showed that reference communities were significantly different from those in contaminated areas as well as separate single compound contaminant plots (RDX and TNT) from mixed compound treatment plots (Comp B). RDX and TNT were not different from one another based on species composition (Table 3.3).

Functional Composition

The number of species representing animal dispersed ($F=3.15$, $p=0.0383$), annual ($F=7.91$, $p=0.0004$), graminoid ($F=6.19$, $p=0.0019$), invasive ($F=3.14$, $p=0.0387$), monocot ($F=5.45$, $p=0.0038$), perennial ($F=3.41$, $p=0.0291$), trailing ($F=3.88$, $p=0.0180$), tree ($F=4.730$, $p=0.00077$), and vine ($F=5.26$, $p=0.0046$) functional groups were significantly different between groups (Table 3.4). RDX plots were most often responsible for the detected differences, exhibiting far more annual ($p=0.0002$), monocot ($p=0.0149$), graminoid ($p=0.0053$), trailing ($p=0.0203$), and vine ($p=0.0034$) species, as well as fewer animal dispersed species ($p=0.0475$) relative to reference. RDX also distinguished itself from TNT and Comp B plots with significantly higher counts of vine (Comp B $p=0.0344$), grass (TNT $p=0.0054$, Comp B $p=0.0125$), annual (Comp B $p=0.0159$), and monocot (TNT $p=0.0076$, Comp B $p=0.0169$) species. Additionally Comp B plots exhibited elevated numbers of tree and invasive species but was only significantly different from TNT plots ($p=0.0036$ and $p=0.0268$ respectively)

Community weighted means (CWM) revealed significant differences across treatments for several functional traits. CWM for species native status exhibited significant reductions ($F=3.59$, $p=0.024$) for TNT relative to RDX ($p=0.029$) and reference ($p=0.045$) plots. A similar trend was observed for life history ($F=3.780$, $p=0.020$) as values for TNT plots were significantly lower than those of RDX ($p=0.042$) and reference ($p=0.027$) ones. TNT plots also exhibited significantly higher CWM values for photosynthetic mechanism ($F=4.76$, $p=0.007$) compared to RDX ($p=0.007$) and reference ($p=0.027$). Despite these differences cluster analysis was unable to produce any distinctive pattern and the MRPP was unable to define the preexisting grouping scheme ($A=-0.004$, $p=0.4766$).

Discussion

Due to the inherent risk of injury at many locations where unexploded ordnance (UXOs) are present, field monitoring of vegetative responses is rare; legacy community level information even more so. As such, the objective of our study was to investigate and quantify long term impacts of explosives soil contamination on a vegetative community. Our initial hypotheses were that 1) community richness and diversity in contaminated areas would not exhibit change from reference plots as species will fill in vacant niches, 2) species composition would differ from reference plots as the presence of contaminants filter out intolerant species, and 3) species functional traits would differ in contaminated plots relative to those of reference. Based on the results presented here all three hypotheses were supported.

Presence or absence of explosives compounds was unable to differentiate treatment groups from one another using standard species diversity metrics (S , H' , D_1). Woch *et al.*, (2016) found a similar response along a heavy metal gradient and suggested that similarity was due to less tolerant species being removed and more tolerant species coming in and establishing in the

newly formed gaps and this trend has also been observed repeatedly in systems under natural disturbance regimes (Bobbink, 1991; Barbaro *et al.*, 2004; Questad and Foster, 2007; Stevens *et al.*, 2010; Kurek *et al.*, 2014). This idea is further supported by the significant increase in annual and monocot species presence in RDX plots as well as the increase in annuals CWM in TNT plots. Such shifts in are common on disturbed sites and thought to have increased tolerance for contaminant presence (Schnoor, *et al.*, 1995; Quist *et al.*, 2003; Winfield *et al.*, 2004; Panz *et al.*, 2013; Zhang and Chu, 2013). There were shifts towards graminoid dominance in RDX plots. Graminoid species are highly resilient to RDX presence (Best *et al.*, 2007); however, graminoids are sensitive to TNT and other explosive mixtures (Peterson *et al.*, 1998; Best *et al.*, 2007). TNT plots showed a novel shift in functional dominance, via CWM values, with the significant increase in C₄ plant presence. This dominance of C₄ species in TNT plots was particularly interesting as TNT induces significant impacts to photosynthetic mechanisms yet C₄ species appear to be more tolerant overall to presence of explosive compounds, and may have led to this shift (Via and Zinnert, 2016).

Simpson's index of similarity showed that species composition was ~60% or less similar for all treatment groups when all species were considered. Given the proximity of the plots (5-50m) and the homogeneity of both soil and terrain the logical cause of this difference was the presence of contaminants. Observed similarities in community composition appeared to be largely driven by herbaceous species as there was little change in index values when woody species were removed from the analysis. In contrast woody species composition exhibited high variance in similarity across groups with values being as low as 14% and as high as 43%. Reduced species similarity and alterations to dominant functional groups among plots in conjunction with a lack of change for species richness and diversity suggest that compositionally

plots were also different across treatment groups. It appeared that the field plots exhibited an altered successional trajectory emphasizing the need to investigate broad scale effects of anthropogenic activities on natural systems.

Community composition produced the clearest differentiation of plots with three distinct groups 1) reference, 2) single contaminant, and 3) multiple contaminant plots. Functionally differences were observed for TNT and RDX in terms of representative species count (Figure 3.4) and CWM values, yet there were no significant impacts for Comp B plots. Similar response patterns have been observed at the individual scale for both morphological and physiological characteristics (Via *et al.*, 2014). TNT appears to influence belowground structures and photosynthetic operation while RDX impacts aboveground structures as well as photosynthetic operation (Via *et al.*, 2014; Via *et al.*, 2015; Zinnert *et al.*, 2013). Unlike the two constituent compounds, Comp B tends to produce reduced impacts, requiring larger concentrations to induce the same level of impairment at the species level. Differences were present for CWM values denoting shifts in dominant traits between treatment groups (Villéger *et al.*, 2008) and potentially altering ecosystem processes (Vile *et al.*, 2006; Mokany *et al.*, 2008).

Small scale impacts of disturbance can have significant direct and indirect influences on larger scale processes (Heffernan *et al.*, 2014). At the individual level explosives have an array of morphological and physiological impacts on vegetation (see Via and Zinnert, 2016) which can influence success and fecundity of colonizing species (Prach, *et al.*, 2014). By limiting intolerant species and providing niches for tolerant ones contaminants act the same way that environmental filters do (Lambers *et al.*, 2008). Considerable effort has been put into understanding short term impacts of anthropogenic contaminants on vegetative health, but more work is needed investigating long term impacts of exotoxins.

Species diversity and composition cannot fully explain ecosystem level impacts of a disturbance. By quantifying functional traits and composition of a community a much more complete understanding of disturbance effects and influences on recolonization trends can be gained (Ding *et al.*, 2012; Letcher, 2010; Letcher *et al.*, 2012; Norden *et al.*, 2012; Whitfield *et al.*, 2012; Feng *et al.*, 2014). From a functional trait perspective there was less distinction among treatment groups, with most shifts occurring in RDX contaminated areas. RDX plots had far more annuals, monocots, graminoids and vines, as well as fewer animal dispersed species. Increases in annuals and graminoids have been observed in the presence of contaminants (Schnoor, *et al.*, 1995; Quist *et al.*, 2003; Zhang and Chu, 2013). Compositional changes away from zoochorous species has been connected with harsh habitats; particularly young or disturbed ones (Řhounková and Prach, 2010; Woch *et al.*, 2016). Functional richness and divergence have often been linked to community assembly processes (Mouchet *et al.*, 2010; Mason *et al.*, 2012; Spasojevic and Suding 2012) or ecosystem functioning (Petchey *et al.*, 2004; Mason *et al.*, 2013; Mouillot *et al.*, 2013).

Our results suggest that long-term impacts of explosives contamination affect community composition and functional traits from chronic leaching of point source toxic compounds over nearly two decades. The “dummy devices” used here were designed and placed in the field to closely mimic anti-personnel landmines allowing for direct comparisons to locations with UXO presence. Given the global distribution of UXOs and the continued use of landmines (Via and Zinnert, 2016) understanding the ecological impacts of these devices is of great importance. Concentration of UXOs at this field site were relatively small suggesting that vegetative community condition on contaminated systems (military training grounds, past and current warzones, industrial landscapes, etc.) may exhibit more significant responses. While UXOs have

devastating impacts to human life and health they also pose a serious ecological risk both from physical damage via explosive potential and from toxic compounds may enter the soil.

Legacy community level impacts of explosive compounds are capable of being identified using various species and functional trait metrics. Presence of explosive compounds had impacts on community species and functional composition yet elicited no response in standard species diversity metrics. RDX and TNT contaminated plots had shifts in dominant functional traits, suggesting an influx of more tolerant species. RDX possessed the most unique combination of functional traits relative to reference and other treatment groups. Comp B had no significant impact on any metric which agrees with findings in recent literature. More long term broad scale investigation of contaminants and disturbance are needed to fully understand anthropogenic impacts on natural systems.

Table 3.1: Mean and standard error of diversity metrics (richness [S], Shannon-Weiner [H'], and Simpson index [D₁] values) for reference and explosives contaminated plots.

| | | Reference | RDX | TNT | Composition B | F | p |
|---------------------------|----------------------|-----------|-----------|-----------|---------------|------|-------|
| All Species | S | 9.90±0.75 | 8.78±0.55 | 8.22±0.66 | 8.63±0.73 | 1.18 | 0.33 |
| | H' | 2.27±0.07 | 2.16±0.06 | 2.08±0.08 | 2.13±0.09 | 1.12 | 0.36 |
| | D₁ | 0.89±0.01 | 0.88±0.01 | 0.87±0.01 | 0.88±0.01 | 1.08 | 0.37 |
| Herbaceous Species | S | 7.30±0.62 | 7.00±0.37 | 6.33±0.53 | 5.75±0.67 | 1.51 | 0.23 |
| | H' | 1.01±0.10 | 1.25±0.07 | 0.78±0.07 | 0.92±0.13 | 4.41 | 0.01 |
| | D₁ | 0.47±0.05 | 0.61±0.03 | 0.35±0.03 | 0.45±0.07 | 5.32 | 0.004 |
| Woody Species | S | 2.78±0.32 | 2.11±0.26 | 2.00±0.19 | 3.13±0.35 | 3.37 | 0.03 |
| | H' | 0.88±0.12 | 0.52±0.13 | 0.55±0.10 | 0.98±0.12 | 3.79 | 0.02 |
| | D₁ | 0.53±0.05 | 0.32±0.08 | 0.36±0.07 | 0.57±0.06 | 3.54 | 0.03 |

Table 3.2: Percent similarity of species composition between treatments according to Jaccard's index for reference and explosives contaminated plots.

| | All Species | | | | Herbaceous Species | | | | Woody Species | | | |
|-----------|-------------|-----|-----|--------|--------------------|-----|-----|--------|---------------|-----|-----|--------|
| | Reference | RDX | TNT | Comp B | Reference | RDX | TNT | Comp B | Reference | RDX | TNT | Comp B |
| Reference | -- | | | | -- | | | | -- | | | |
| RDX | 51 | -- | | | 61 | -- | | | 14 | -- | | |
| TNT | 50 | 50 | -- | | 59 | 62 | -- | | 29 | 17 | -- | |
| Comp B | 44 | 46 | 60 | -- | 48 | 48 | 59 | -- | 29 | 43 | 33 | -- |

Table 3.3: P-values from pairwise comparisons of species and functional composition of treatment groups according to multi response permutation procedure (MRPP) analysis for reference and explosives contaminated plots. Bold text indicates the presence of a significant difference ($p < 0.05$).

| | Species | | | | Functional Type | | | |
|---------------|---------------|---------------|---------------|--------|-----------------|--------|--------|--------|
| | Reference | RDX | TNT | Comp B | Reference | RDX | TNT | Comp B |
| Reference | -- | | | | -- | | | |
| RDX | 0.0013 | -- | | | 0.5425 | -- | | |
| TNT | 0.0003 | 0.0822 | -- | | 0.3363 | 0.2741 | -- | |
| Composition B | 0.0014 | 0.0011 | 0.0074 | -- | 0.6403 | 0.2840 | 0.6207 | -- |

Table 3.4: Mean and standard error of species count in each treatment for various functional types for reference and explosives contaminated plots. Letter codes designate statistical grouping. Rows lacking letter codes have significant differences present. Bold text signifies a significant difference from reference values.

| | | Reference | RDX | TNT | Comp B |
|---------------------|--------------------|--------------------------|--------------------------------|----------------------------|----------------------------|
| Life History | <i>Annual</i> | 0.90 ± 0.23 ^a | 2.56 ± 0.30^b | 1.78 ± 0.22 ^a | 1.38 ± 0.26 ^{a,b} |
| | <i>Biennial</i> | 1.80 ± 0.33 | 1.33 ± 0.24 | 1.33 ± 0.17 | 1.00 ± 0.27 |
| | <i>Multiannual</i> | 8.90 ± 0.67 | 6.56 ± 0.58 | 6.44 ± 0.67 | 7.75 ± 0.82 |
| | <i>Perennial</i> | 7.10 ± 0.57 | 5.20 ± 0.55 | 5.11 ± 0.56 | 6.75 ± 0.56 |
| Dispersal Mechanism | <i>Zoochory</i> | 5.70 ± 0.73 ^a | 3.56 ± 0.15^b | 3.78 ± 0.57 ^{a,b} | 4.75 ± 0.45 ^{a,b} |
| | <i>Ballochory</i> | 0.20 ± 0.13 | 0.22 ± 0.15 | 0 ± 0 | 0.13 ± 0.13 |
| | <i>Hydrochery</i> | 0 ± 0 | 0.11 ± 0.11 | 0 ± 0 | 0 ± 0 |
| | <i>Anemochory</i> | 3.90 ± 0.43 | 5.22 ± 0.32 | 4.44 ± 0.41 | 4.25 ± 0.37 |
| Cotyledon | <i>Dicot</i> | 7.90 ± 0.77 | 6.11 ± 0.46 | 6.56 ± 0.71 | 7.25 ± 0.59 |
| | <i>Monocot</i> | 1.80 ± 0.30 ^a | 3.00 ± 0.46^b | 1.67 ± 0.17 ^a | 1.75 ± 0.37 ^a |
| Vegetation Type | <i>Fern</i> | 0.10 ± 0.10 | 0 ± 0 | 0 ± 0 | 0.13 ± 0.13 |
| | <i>Forb</i> | 0.60 ± 0.22 | 1.00 ± 0.17 | 1.33 ± 0.37 | 0.50 ± 0.19 |
| | <i>Graminoid</i> | 1.70 ± 0.26 ^a | 3.00 ± 0.24^b | 1.67 ± 0.17 ^a | 1.75 ± 0.37 ^a |
| | <i>Herbaceous</i> | 2.70 ± 0.50 | 2.22 ± 0.15 | 2.00 ± 0.24 | 1.63 ± 0.50 |

| | | | | | |
|----------------|--------------------|----------------------------|--------------------------------|----------------------------|----------------------------|
| Seed Size | <i>Tree</i> | 2.63 ± 0.26 ^{a,b} | 1.80 ± 0.33 ^{a,b} | 1.78 ± 0.28 ^a | 1.11 ± 0.20 ^b |
| | <i>Vine</i> | 2.63 ± 0.18 ^a | 3.00 ± 0.57^b | 1.11 ± 0.35 ^{a,b} | 2.11 ± 0.35 ^{a,b} |
| | <i>Small Seed</i> | 1.40 ± 0.34 | 1.40 ± 0.24 | 1.22 ± 0.67 | 1.75 ± 0.53 |
| | <i>Medium Seed</i> | 5.70 ± 0.54 | 6.20 ± 0.43 | 5.44 ± 0.67 | 5.00 ± 0.50 |
| | <i>Large Seed</i> | 2.70 ± 0.50 | 1.44 ± 0.41 | 1.55 ± 0.41 | 2.38 ± 0.18 |
| Native Status | <i>Native</i> | 7.80 ± 0.68 | 7.22 ± 0.52 | 7.20 ± 0.56 | 6.75 ± 0.70 |
| | <i>Invasive</i> | 2.00 ± 0.26 | 1.89 ± 0.35 | 1.22 ± 0.22 | 2.38 ± 0.18 |
| Growth Habit | <i>Erect</i> | 5.60 ± .56 | 6.44 ± 0.60 | 5.40 ± 0.56 | 5.75 ± 0.75 |
| | <i>Prostrate</i> | 0.80 ± 0.45 | 0.89 ± 0.2 | 0.67 ± 0.17 | 0.50 ± 0.27 |
| | <i>Trailing</i> | 3.40 ± 0.4 ^a | 1.78 ± 0.47^b | 2.11 ± 0.39 ^{a,b} | 2.88 ± 0.13 ^{a,b} |
| Root Structure | <i>Taproot</i> | 6.60 ± 0.72 | 5.78 ± 0.34 | 5.89 ± 0.56 | 6.25 ± 0.68 |
| | <i>Fibrous</i> | 3.20 ± 0.36 | 3.33 ± 0.33 | 2.33 ± 0.29 | 2.88 ± 0.48 |

Figure Legends

Figure 3.1: Location of field sites in Edgefield County, South Carolina. Colored circles show field plots where data was collected. Red marks reference plots, yellow marks RDX plots, blue TNT, and green Composition B.

Figure 3.2: Woody species density across treatment types for reference and explosives contaminated plots. Letters denote statistical grouping. The lack of letter codes signify that no difference was present between treatments.

Figure 3.3: Dendrogram of plots based on species composition for reference and explosives contaminated plots. Axis along the top represent information remaining and the red dashed line represents the cutoff point used here.

Figure 3.4: Graphical representation of community shifts across treatment area.

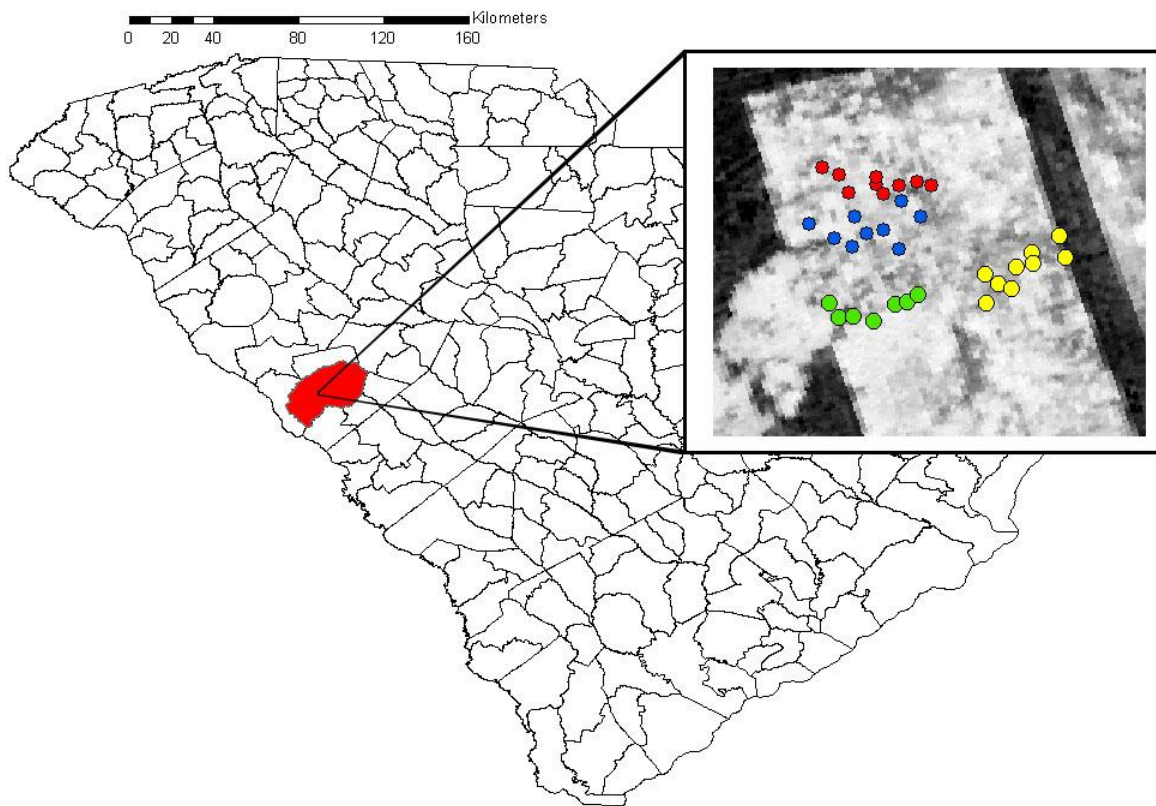


Figure 3.1

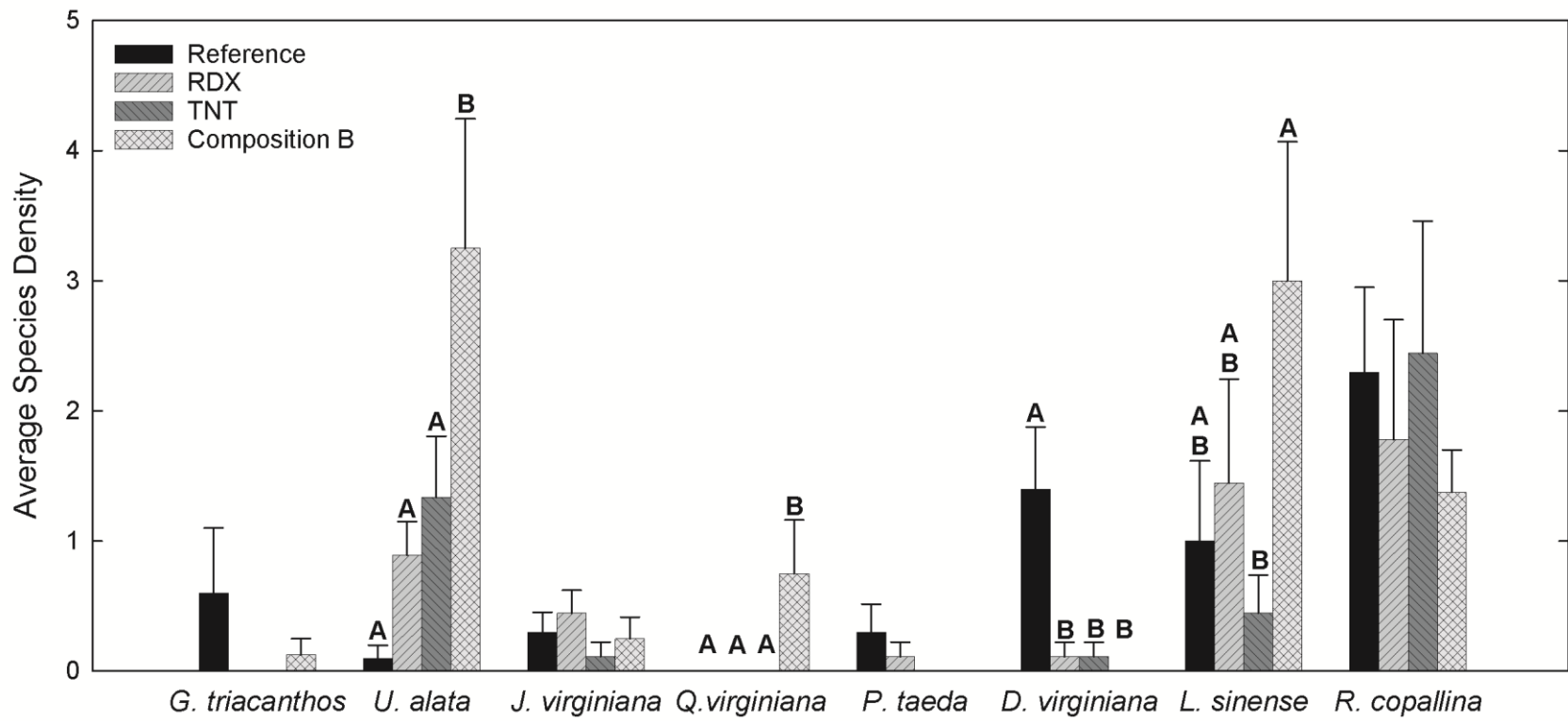


Figure 3.2

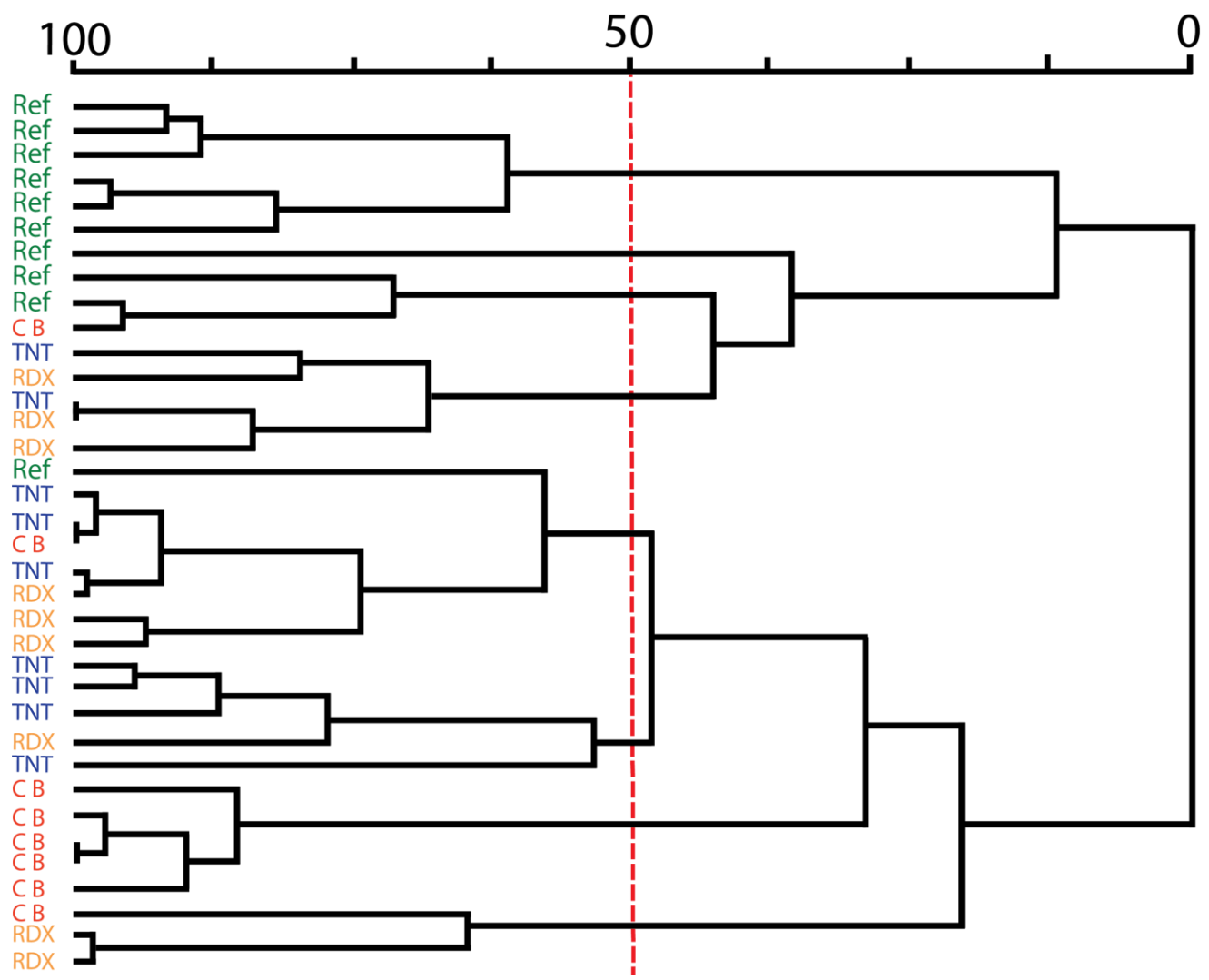


Figure 3.3

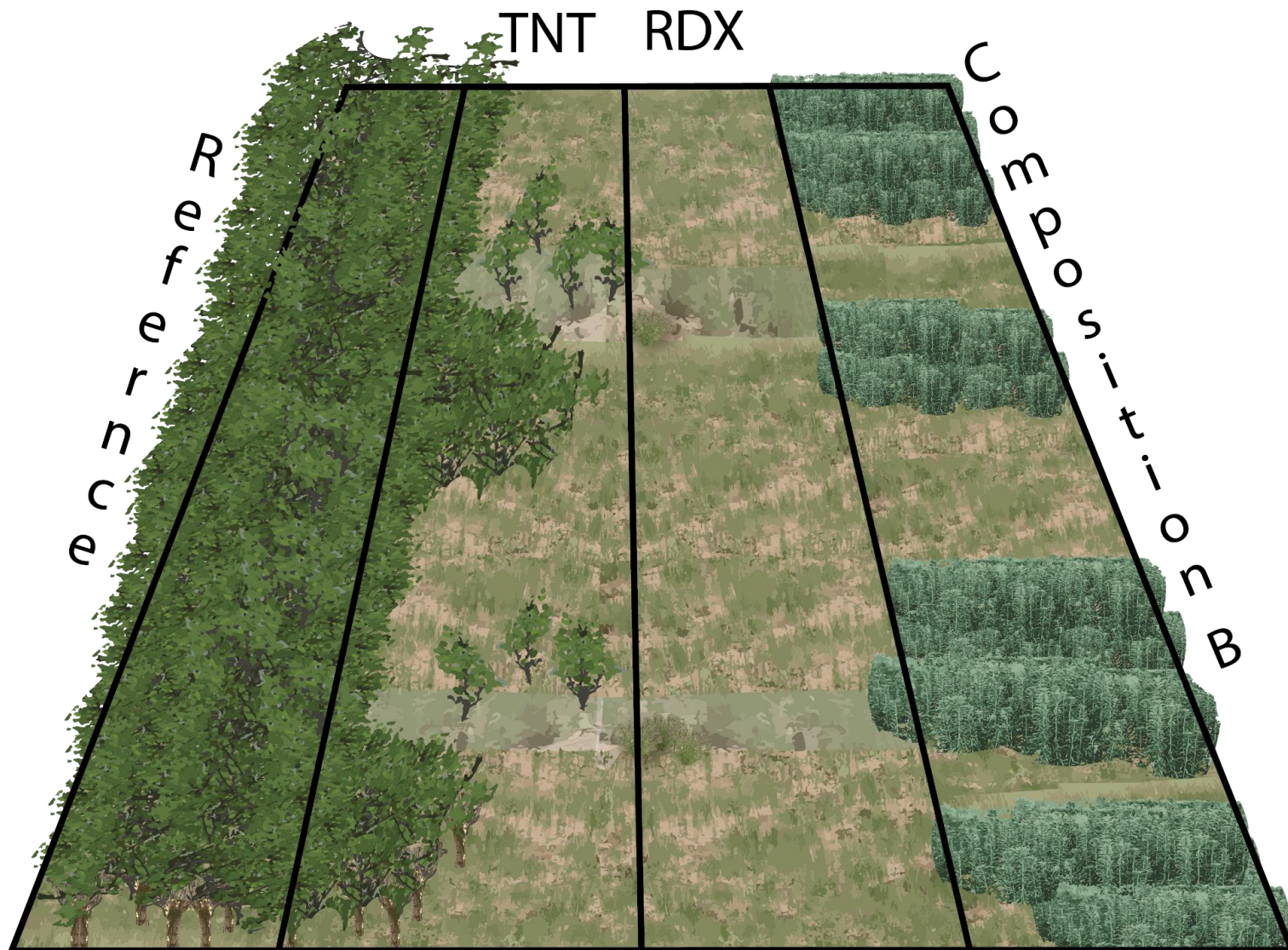


Figure 3.4

Chapter 4

Linking Physiological and Spectral Impacts of Explosives Soil Contamination on Vegetation

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Introduction

Explosive compounds are present on a large portion of the globe (UNICEF, 1995; Pichtel, 2012; Via and Zinnert, 2016) due to military, industrial, and civilian use (Myler and Sisk, 1991; Kholodenko *et al.*, 2014). Military and associated industries are the predominant contributor of environmental explosives contamination (Best *et al.*, 1999; Just and Schnoor, 2004; Pichtel, 2012; Certini *et al.*, 2013). Unexploded ordnances (UXOs) from conflicts and training activities are a hazard to both human and environmental health. Aside from the inherent risk of detonation, there is the threat of contaminant leaching (Taylor *et al.*, 2015). Explosive compounds radiate out from the contaminant source, moving in solution through the soil pore matrix (Pennington and Brannon, 2002; Kiiskila *et al.*, 2015). Eventually these compounds may be absorbed by vegetation via bulk flow of water (Pilon-Smits, 2005). While many explosive compounds have been in use, the most common organic explosive compounds found in ordnance are RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and TNT (2-methyl-1,3,5-trinitrobenzene; Rylott and Bruce, 2008; Pichtel, 2012).

RDX and TNT compounds enter plants the same way, but are structurally very different and have distinct impacts on vegetation (reviewed in Via and Zinnert, 2016). RDX, a nitroamine compound, bioaccumulates in aboveground plant tissues (Ahmadi *et al.*, 1980; Thompson *et al.*, 1999; Best *et al.*, 2006), while TNT, a nitroaromatic compound, is primarily bound within root tissues (Ahmadi *et al.*, 1980; Winfield *et al.*, 2004; Pilon-Smits, 2005). Sequestered compounds are stored in vacuoles or bound in cellulose and lignin complexes (Burken *et al.*, 2000; Lotufo *et al.*, 2009; Rylott *et al.*, 2011; Schoenmuth *et al.*, 2015) and are capable of inducing a variety of physiological and morphological responses (Via and Zinnert, 2016). RDX causes significant deformation and reduction in function of aboveground parts of the plant (Winfield *et al.*, 2004)

including: curled or irregular leaf margin, fused leaves, bifurcated leaves, atypical pigmentation, reduced leaf chlorosis, and necrotic lesions (Winfield *et al.*, 2004; Vila *et al.*, 2007). TNT impairs growth and function of belowground tissues, inhibiting the formation of new roots and destroying existing primary and secondary root structures (Gong *et al.*, 1999; Comfort *et al.*, 1998; Peterson *et al.*, 1998; Khatisashvili *et al.*, 2009).

RDX and TNT influence physiological processes, significantly hampering photosynthetic function as well as water relations (Ait Ali *et al.*, 2006; Zinnert, 2012; Via *et al.*, 2014). Although the responses can be significant for both compounds, they are not always similar. Both explosives cause significant reductions in photosynthesis, especially with regard to altered leaf fluorescence; however, RDX increases stomatal conductance while TNT inhibits gas exchange through the stomata (Via *et al.*, 2014). These morphological and physiological responses to the presence of explosive compounds are important not only in terms of understanding impacts of these contaminants but also as a method for detecting the presence of explosive compounds in the soil.

A number of technologies exist for field detection of UXOs ranging from x-ray radiography, to ultrasound and sonar, to thermography (Bello, 2013); however, these systems can only detect UXOs at a relatively close range and in some cases require human operators to endanger themselves. Using broad-scale remote sensing to detect the presence of UXOs has been investigated (Clark *et al.*, 1995; Robinson and Ruff, 1997; Clark *et al.*, 2000; Tiwari *et al.*, 2008; Bajić *et al.*, 2013), but much like the other methodologies one factor continued to be a hurdle, the presence of vegetation. Vegetation impairs the ability to discern the presence of UXOs by blocking signals from the most commonly used sensors, making vegetated areas difficult to clean of explosives and undergo remediation.

Vegetation stress is accompanied by known shifts in cellular processing and pigment content (Fang *et al.*, 1998). These shifts can be detected and monitored via stand-off remote detection (Zinnert *et al.*, 2012). Hyperspectral remotely sensed data provide very detailed information about how vegetation reflects light, which can be used to infer plant health (Blackburn, 2007). Explosives compounds are known to induce changes in vegetation reflectance spectra, particularly in the visible region (400-800 nm) which corresponds to morphological and physiological damage (Naumann *et al.*, 2010; Zinnert, 2012). Hyperspectral reflectance may be of use in monitoring degraded or disturbed locations.

The objective of this study was to determine if soil contaminated with explosives over a decade impacts plant physiology of dominant woody vegetation and to investigate the use of hyperspectral imagery to detect such contamination. Specifically, we wanted to determine if impacts to electron transport rate (as a proxy for photosynthetic potential) and hydraulic conductivity (i.e. plant water status) were impaired on two dominant canopy species. Building upon the extensive foundation of knowledge surrounding plant responses to stressors with remote sensing tools we also investigated potential shifts present in plant reflectance spectra and subsequently shifts in standard vegetation indices. To our knowledge, this is the first study to investigate vegetation impacts of explosives contaminated soil on plant physiology and reflectance in a relic field site over a decade post contaminant placement.

Methods

Site Description

Imagery and physiology data were collected on a privately owned experimental minefield in Edgefield, SC, USA (33°54'5.39"N, 82° 2'1.79"W; Figure 4.1). In 1999 the test area was cleared of all vegetation and sectioned into 5 x 5 m plots. Within a number of those plots

explosive compounds were buried in “dummy mines” designed to mimic antipersonnel mines containing roughly 75 – 125 g of explosives. The dummy mines were cardboard boxes filled with TNT, RDX, and a mixture of the two compounds; Composition B (Comp B; consisting of 60% RDX and 40% TNT). Reference areas were delineated upslope of the contaminated plots in the razed area. Two target species, *Ulmus alata* (Michx.) and *Rhus copallina* (L.), were the most common woody species present at the field site with canopies large enough for use with the hyperspectral imagery (Via *et al.*, in press). Locations of individuals of each species across reference and treatment plots were marked (n=3) with a GPS for use in airborne imagery analysis.

Plant Physiology and Uptake

Physiological measurements were collected on mature *U. alata* and *R. copallina* individuals concurrently with airborne image acquisition on September 19th 2011 at midday (1100-1300). Relative light curves were recorded for individuals of each species in the field, from which electron transport rate (ETR) values were obtained at each time-stop. Fluorescent measurements were recorded using a pulse amplitude modulated fluorometer (Mini-PAM, Walz, Germany). Plant water relations were quantified as native stem hydraulic conductivity (kh; kg m⁻¹ s⁻¹ MPa⁻¹) and leaf specific conductivity (LSC; kg m⁻¹ s⁻¹ MPa⁻¹) which were measured from branches clipped at midday in the field (n = 5). Clipped branches were sealed in bags, transported to the laboratory, and processed as explained in Sperry *et al.*, (1988) using a custom setup consisting of an IV-bag supplying filtered (0.2 µm) 20 mM KCl solution to a stem segment using low gravitational pressure (~5 kPa). A low hydraulic head was used to prevent removal of embolisms and flow rate was monitored using an analytical balance (Model PA64, Ohaus), connected to a computer. Hydraulic conductivity was calculated as mass flow rate of

solution through the clipped stem segment divided by the pressure gradient along the segment path length ($kh, \text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$).

Leaf samples were collected in the field and dried for later analysis. Extraction of RDX and TNT was accomplished using a modified version of methods specified in EPA SW-846 Method 8330 (USEPA, 1989; Larson *et al.*, 1998). Pre-weighed tissue samples were placed glass vials (20 ml) with Teflon-lined caps with 10 mL of high performance liquid chromatography (HPLC) grade acetonitrile. Samples were vortexed for ~1 min prior to being placed in a cool ultrasonic bath for 18 h (USEPA, 1989). Following sonication extracts were allowed to settle for one hour, after which 2.5 ml of the supernatant was filtered through a clean-up column containing 0.25 g of Florisil and 0.25 g of basic alumina which had been wetted with acetonitrile first to remove chlorophyll pigments that could interfere with UV detection of RDX and TNT. Next, 2.5 ml of HPLC grade acetonitrile was passed through the same column and mixed with the supernatant. A sample of the supernatant was diluted to 1:1 (v/v) with deionized (DI) water and filtered into an amber HPLC vial via a disposable 10 ml Luer-Lok syringe with a 0.45 μm Teflon filter.

Leaf extracts of both species were analyzed for explosives related compounds using a Thermo Scientific Dionex Ultimate 3000 HPLC system with rapid separation auto sampler and UV detector set at 245 nm. The columns used were Thermo Scientific Acclaim Explosives Columns E1 (LC-18) and E2 (CN) using a 50/50 (v/v) methanol/water mixture for the mobile phase with a flow rate of 1.0 mL min^{-1} and an injection volume of 25 μL . A seven point calibration curve was generated to quantify the RDX and TNT in plant samples and calibration standards of the compounds were prepared in acetonitrile/water (50:50 v/v) from stock solution (100 mg L^{-1}) at levels of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 $\mu\text{g s}^{-1}$.

Airborne Hyperspectral Acquisition

Hyperspectral data were collected by SpectIR (SpectIR Corp., Reno, NV, USA) using the ProSpecTIR VIS hyperspectral imaging spectrometer mounted in a Cessna 207 aircraft. Spatial resolution of the data was 1 m²/pixel, with a spectral range of 400–2500 nm, producing 360 bands, with a signal to noise ratio of 884:1. Data collection occurred on the 19th of September in 2011 under cloud-free conditions. Data were received post-processed to minimize geometric and radiometric effects. Ground reflectance radiometry was used to calibrate the data based on target endmembers collected in-scene with the ASD FieldSpec Pro Full Range reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO, USA). This effectively placed the scene into reflectance units. The resulting image was 16 bit signed projected in UTM 17 Northern Hemisphere using Clarke 1866 geographic coordinate system.

The original scene was clipped to the test area in ERDAS Imagine (Hexagon Geospatial) and subsequently converted into a double floating point raster. Using the resulting raster site-wide index maps were generated using model builder for 14 different industry standard spectral indices related to changes in pigment content/concentration, photosynthetic function, biomass, as well as nutrient and water status of vegetation (Table 4.1). Image files were then loaded into ArcMap (ESRI) and pixel values extracted at field recorded GPS locations of the target species using the extract values to points tool. Spectral profiles were also obtained for each plot using the spectral analysis tool in ERDAS Imagine.

Due to the close proximity of some individuals of both species it was not possible to discriminate canopies in the spectral data. This resulted in limited sample sizes for *U. alata* in Comp B areas and *R. copallina* in RDX areas preventing analysis of individuals of these species for those treatment plots.

Statistical Analyses

U. alata and *R. copallina* values for all metrics were analyzed separately to prevent bias being introduced in the data from inter-species variation. ETR data obtained from relative light curves were analyzed using repeated measure analysis of variance (ANOVA) followed by ANOVA of ETR values at each time-step. Hydraulic conductivity and hyperspectral index values were analyzed via ANOVA across treatment groups, followed by Tukey's *post-hoc* tests. Multivariate analysis of treatment grouping was conducted via principal components analysis (PCA) using covariance as the distance metric due to the scale of the data, for both raw spectral and index values. Spectral and index values were also analyzed for treatment grouping using multi-response permutation procedure (MRPP) analyses with covariance as the distance.

Data were analyzed using JMP 13.0 (SAS) and SAS 9.4 (SAS).

Results

Plant Physiology and Uptake

Light response curves showed that changes in both species were present in contaminated areas (Figure 4.2). *Ulmus alata* had significant reductions in electron transport rate (ETR) in treatment plots compared to reference ($F=3.81$, $p=0.021$). TNT individuals exhibited significant reductions from $\sim 150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p=0.0174$) to $\sim 600 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p=0.6116$). Comp B individuals were also distinct from reference between $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p=0.0386$) and $\sim 300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p=0.0518$). *Rhus copallina* individuals exhibited significantly reduced ETR, relative to reference, with increased PAR with regard to treatment ($F=31.12$, $p<0.0001$). Individuals in TNT contaminated areas had significantly reduced ETR values beginning at $\sim 150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p<0.0001$) and continuing through all higher PAR levels. RDX also exhibited significant reductions in ETR from $587 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p=0.039$) until $1305 \mu\text{mol m}^{-2} \text{s}^{-1}$

($p=0.543$). No significant changes were present for *R. copallina* Comp B contaminated areas when compared to reference plots ($p>0.05$).

There was no effect of treatment for stem hydraulic conductivity in either *U. alata* ($F=0.30$, $p=0.8826$) or *R. copallina* ($F=0.1918$, $p=0.9010$; Table 2). There was a significant change to leaf specific conductivity for *U. alata* ($F=3.07$, $p=0.0474$) in Comp B plots only ($p=0.0348$). No significant differences were found in leaf specific conductance for *R. copallina* in any treatment ($F=1.38$, $p=0.2715$; Table 4.2).

Compounds associated with RDX were detectable in leaf tissues from RDX and Comp B plots (Table 3). *Ulmus alata* samples possessed the greatest variety of explosive related compounds having all five compounds tested for present in leaf tissues while only two were at detectable levels in *R. copallina* tissues. TNX concentrations were significantly high in *U. alata* and *R. copallina* ($F=11.45$, $p<0.0001$; $F=12.02$, $p<0.0001$, respectively) located in RDX and Comp B areas. Significantly high levels of DNX were observed for *U. alata* ($F=3.26$, $p=0.0374$); however, Tukeys *post-hoc* test failed to identify the cause of the difference (Table 3). Despite *U. alata* individuals in RDX plots also contained MNX, HMX, and RDX itself the levels found in the leaf tissues were not significantly elevated (Table 4.3). This was also true for Comp B *U. alata* individuals which had detectable, but insignificant levels of DNX and MNX present. The only compounds detected in *R. copallina* individuals other than TNX was DNX in RDX contaminated areas (Table 4.3).

Hyperspectral Data

Spectral profiles of species in contaminated areas exhibited reductions in reflectance values across large portions of visible and infrared regions (Figure 3). Principal components analysis (PCA) was able to differentiate treatment groups for both *U. alata* and *R. copallina*.

Ulmus alata spectra exhibited tight clustering of reference plots and decent separation of treatment groups. Axis 1 was the predominant source of group separation (explaining 82.09% of the variation; Figure 4) with reference separating from contaminant individuals, and the TNT and RDX values separating from one another. Axis 2, which explained 13.23% of the variation, did separate reference from treatment values as well; however, was unable to distinguish between the two contaminant groups. For *R. copallina* Axis 1 and 2 explained 91.18% of the variation observed (66.68% and 24.51% respectively; Figure 4). Reference individuals were separated from those in Comp B and TNT areas by both Axes 1 and 2, while the contaminant treatments themselves were separated solely by Axis 1 (Figure 4).

Multi-response permutation procedure (MRPP) analysis for *U. alata* spectra defined the preexisting grouping scheme ($A=0.78$, $p=0.009$). There was distinct delineation between reference from TNT ($p=0.022$) and Comp B ($p=0.026$), as well as TNT from Comp B groups ($p=0.032$). Interestingly the MRPP for *R. copallina* was unable to differentiate all groups based on spectral profiles ($A=0.35$, $p=0.0690$), but the pairwise comparison did show that reference and TNT groups were distinguishable from one another based on their spectra ($p=0.047$). Reference and Comp B were not differentiated from one another ($p=0.085$) nor TNT from Comp B ($p=0.537$).

Of the 14 indices tested here (Table 1), all exhibited significantly different values for contaminated locations relative to references (Table 4). The indices which exhibited significant shifts were predominantly associated with changes in leaf pigments (Carotenoid R1, Simple Ratio indices, VOG1, and Greenness). Indices telling of water relations, biomass, nutrition, and photosynthesis also revealed significant changes (SIPI, Sum Green, NDVI, PSRI, and Moisture Stress). *Ulmus alata* index values showed significant changes in RDX and TNT areas. *Rhus*

copallina only showed significant changes in the TNT plots. Not only did the two species differ somewhat in which indices exhibited significant shifts in the presence of the contaminants, *U. alata* also had significant differences across more indices than *R. copallina* (Table 4.4). Interestingly Only *R. copallina* individuals in TNT areas expressed significant shifts in water related indices. Overall there was no separation of RDX and TNT index values for *U. alata*. In *R. copallina*, Comp B and TNT followed a similar trend with all index values being statistically similar with the exception of Carotenoid R1 and WBI indices (Table 4.4).

Single indices can be very helpful for investigating plant health but each index is susceptible to limitations and is not able to provide a holistic perspective of plant health and function. Multivariate approaches comparing multiple indices simultaneously should be used to account for the limitations of a single index. Taking into consideration all tested vegetation indices for *U. alata* via PCA provided separation of treatment groups, although lacked tight clustering of individuals for each treatment (Figure 4.5). The first two axes explained 94.32% of the variations (78.13 and 16.19% respectively). *Rhus copallina* index values provided better delineation of groups than those for *U. alata*. Axis 1 explained 86.42% of the variance clearly separating reference from contaminated individuals (Figure 4.5). Axis 2, which explained only 5.84% of the variance, was responsible for showing separation between TNT and Comp B contaminated individuals.

MRPP analyses for showed that index values of *U. alata* defined the preexisting grouping scheme ($A=0.557$, $p=0.0148$); particularly with regard to differentiating reference from TNT groups ($p=0.0259$). Reference and TNT were not differentiated from RDX based on index values ($p=0.71$ and $p=0.0626$ respectively). *Rhus copallina* indices were unable to define the groupings scheme ($A=0.516$, $p=0.051$), as was the case for the spectra itself. Pairwise comparison did show

that there was differentiation between indices for reference and TNT ($p=0.022$), but not for reference or TNT and Comp B ($p=0.296$ and $p=0.068$ respectively).

Discussion

The study objective was to investigate the spectral reflectance changes in woody vegetation growing in explosives contaminated soils and relate those changes to physiological shifts observed on the ground. *Ulmus alata* individuals in particular were more sensitive than *R. copallina* to the presence of explosives. *Ulmus alata* responded with reductions to both photosynthetic operation (ETR) and hydraulic conductivity, but *R. copallina* exhibited greater inhibition to photosynthetic processes. Plants in TNT contaminated areas expressed the most drastic shifts in physiology, particularly with regard with ETR. This observed response in TNT contaminated plots is likely due to TNT impacting root structures and causing cascades of stress responses in the vegetation (Schaffer and Ploetz, 1989; Contador *et al.*, 2015). Direct impacts to the photosystems is unlikely given the lack of TNT related compounds in leaf tissues. High performance liquid chromatography (HPLC) did not detect all of the transformation products of biotic metabolism. Therefore, it is possible that compounds originating from TNT, such as formaldehyde and oxygen radicals, could be affecting leaf function (Spain, 1995; Hawari *et al.*, 2000; Halasz *et al.*, 2002; Bernstein and Ronen, 2012). Impacts on vegetation health 12 years after the placement of point sources is both interesting and a concern in that these compounds, even in relatively small concentrations, can be an environmental threat for many years.

Although the exposure period in this study is far longer than any lab based studies which have dominated the plant-explosives interaction literature (reviewed in Via and Zinnert, 2016), responses seen here correspond with those of previous studies. TNT tends to cause greater reductions in photosynthetic function relative to RDX and CompB (Zinnert *et al.*, 2013; Via *et*

al., 2014), RDX and associated compounds are allocated to and sequestered in leaf tissues, while TNT ones are not (Burken *et al.*, 2000; Lotufo *et al.*, 2009; Rylott *et al.*, 2011; Schoenmuth *et al.*, 2015), and spectral shifts for pigments and water relations are detectable (Naumann *et al.*, 2010; Zinnert, 2012; Manley, 2016). Presence of contaminant and transformation products in leaf tissues was particularly interesting given the deciduous nature of these species and the length of time since the point sources were placed in the field (Larson *et al.*, 2008; Taylor *et al.*, 2009). Having gone through periods of senescence and regrowth for over a decade, the presence of degradation compounds in the leaves of both *U. alata* and *R. copallina* demonstrates that contaminants are still leaching from the dummy mines and compounds are being absorbed by surrounding vegetation. The continued availability of these compounds long after initial placement of the dummy mines highlights the long term influence that abandoned munitions can have on surrounding biota.

As expected from other studies, the only compounds detected in leaf tissues were those related to RDX and not TNT as TNT tends to bind to root tissues (Ahmadi *et al.*, 1980; Winfield *et al.*, 2004; Pilon-Smits, 2005), but surprisingly, the concentrations of the compounds in leaves were higher than would be expected (Via *et al.*, 2014). Taylor *et al.*, (2015) found that soil underneath *in situ* 60 mm mortar rounds, which contain ~200-300 g of explosives (VOP-026 Šternberk, s.p., 2011a, b), had explosive compound concentrations ranging from 1 - 10 mg kg⁻¹. As the point sources here contained 75-125 g of explosives finding >1 mg kg⁻¹ of explosives in leaf tissues suggests that these two species are not only sequestering the contaminants but potentially biomagnifying them. These plants may be acting as both a pump and a sink, absorbing and storing a relatively large amount of explosives. This, in conjunction with the deciduous nature, can pose a significant problem to surrounding biota. Plants actively pump and

then deposit via leaf drop contaminants from beneath the soil surface onto the top of the leaf litter layer for microbes, insects, and various other fauna to come into contact (Fellows *et al.*, 2006; Newman and Clements, 2007).

Spectral data and vegetation indices provided an alternative perspective on the state of vegetation health. While there were differences in the spectral profiles, the spectra for both species in reference plots exhibited stress-like patterns while treatment profiles match more closely to what is considered the standard spectral profile shape for healthy vegetation (Govender *et al.*, 2007). Increase of reflectance in blue and red regions as well as a shift in the red edge were key indicators of stress in reference plots (Carter and Knapp, 2001; Blackburn, 2007). This may be due to increase soil reflectance in reference plots and/or the timing of the data collection. Leaf senescence in this region begins in September when the data were collected. Interestingly, no stress was detectable via physiology in reference individuals presented here, suggesting that pigment shifts may be causing bias in the vegetation indices as the plants themselves are still physiologically healthy. A laboratory study including *U. alata* showed significant reductions in C:N and $\delta^{15}\text{N}$ in the presence of Comp B (Via *et al.*, in press). This indicates that plants exposed to explosives contain more nitrogen per unit carbon and utilize a different nitrogen source (potentially from the nitrogen containing explosive material). Higher nitrogen content will affect pigmentation and corresponding reflectance spectra (Merzlyak *et al.*, 2007). These factors should be taken into consideration when attempting to develop detection methods for UXOs via vegetation using spectral data.

All three contaminants were distinguishable based on spectral profiles via principal components analysis (PCA) from airborne acquired imagery. Reference individuals for both species were clearly delineated from contaminant ones; however, the two contaminant groups

were not always distinguishable. Multi-response permutation procedure (MRPP) showed that TNT was capable of separating apart from reference groups but little separation was observed otherwise, despite reductions in the spectral profiles themselves. Indices, as well, failed to distinguish all treatment groups. Largely only TNT contaminated areas were differentiated, although *U. alata* individuals in RDX plots exhibited significantly altered values for some indices. While there were slight changes in physiology for RDX and Comp B, these changes were undetectable via reflectance indices. This is surprising as RDX induces significant morphological damage to vegetation particularly with regard to pigment content and physical changes to leaf shape and size (Winfield *et al.*, 2004; Vila *et al.*, 2007; Via *et al.*, 2014) which was expected to be detected via indices. No index was able to differentiate Comp B from reference values.

There are a variety of UXO detection methods and instrument available today but current detection methods for landmines and UXOs are labor intensive and put lives of animals and humans at risk (Hussein *et al.*, 2000). Methods which allow the human element to be kept at a distance require vegetation to be absent as it can interfere (Seigel, 2002; Xiang *et al.*, 2003). The present results suggest that it may be possible to use aerial or satellite mounted sensors to detect UXO affected vegetation, removing the need for humans or animals to enter potentially hazardous areas. With such a large portion of the world impacted by explosive contamination and UXO presence there are numerous highly vegetated and forested areas demanding a need for detection of contaminants. Through a solid understanding of leaf level processes remote detection of explosives via vegetation becomes a practical possibility (Naumann *et al.*, 2010; Zinnert, 2012).

Table 4.1: Vegetation index information including index name, ID code, equation, and what the index is indicative of. All indices listed below were used in this project and calculated using the equations listed.

| Index Name | ID Code | Equation | Indicative of |
|--|----------------|---------------------------------|----------------------|
| Carotenoid Index 1 | Carotenoid R1 | $(1/R510) - (1/R550)$ | Carotenoids |
| Simple Ratio | F680_630 | $R680/R630$ | Chlorophyll |
| Simple Ratio | F740_630 | $R740/R630$ | Chlorophyll |
| Simple Ratio | F740_685 | $R740/R685$ | Chlorophyll |
| Simple Ratio | F750_710 | $R750/R710$ | Chlorophyll |
| Greenness Index | GI | $R554/R677$ | Chlorophyll |
| Vogelmann Red Edge Index 1 | VOG1 | $R740/R720$ | Chlorophyll |
| Photochemical reflectance index | PRI | $(R531 - R570) / (R531 + R570)$ | Photosynthesis |
| Normalized Difference Veg Index | NDVI | $(R900 - R680) / (R900 + R680)$ | Biomass |
| Plant Senescence Reflectance Index | PSRI | $(R680 - R500) / R750$ | Nutrition |
| Structure-Insensitive Pigment Index | SIPI | $(R800 - R445) / (R800 - R680)$ | Nutrition |
| Moisture Stress Index | MSI | $R1599/R819$ | Water Status |
| Summation of Green Reflectance | Sum Green | $\sum 570 \text{ to } 590$ | Water Status |
| Water Band Index | WBI | $R970/ R900$ | Water Status |

Table 4.2: Mean K_h ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and LSC ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) with standard error for *U. alata* and *R. copallina* across treatments. Bold text indicates a significant difference from reference values.

| | | Reference | TNT | RDX | CB |
|---------------------|-------|-----------------|------------------------|-----------------|-----------------|
| <i>U. alata</i> | K_h | 1.1421 ± 0.1487 | 1.0530 ± 0.2241 | 0.8833 ± 0.2533 | 1.0278 ± 0.2583 |
| | LSC | 0.0005 ± 0.0001 | 0.0009 ± 0.0003 | 0.0006 ± 0.0002 | 0.0015 ± 0.0005 |
| <i>R. copallina</i> | K_h | 2.6402 ± 0.7849 | 3.3104 ± 1.2515 | 2.7601 ± 0.8302 | 3.6680 ± 1.1669 |
| | LSC | 0.0027 ± 0.0010 | 0.0059 ± 0.0025 | 0.0027 ± 0.0011 | 0.0075 ± 0.0031 |

Table 4.3: Mean contaminant concentrations with standard error across treatments (mg Kg⁻¹) for *U. alata* and *R. copallina*. Letter codes indicate statistical grouping of values.

| | | TNX | DNX | MNX | HMX | RDY |
|-----------------------|------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <i>Ulmus alata</i> | Reference | nd ^a | nd ^a | nd ^a | nd ^a | nd ^a |
| | TNT | nd ^a | nd ^a | nd ^a | nd ^a | nd ^a |
| | RDY | 1.20 ± 0.19 ^b | 1.02 ± 0.21 ^a | 0.03 ± 0.02 ^a | 0.71 ± 0.32 ^a | 0.09 ± 0.05 ^a |
| | Comp B | 1.42 ± 0.19 ^b | 0.13 ± 0.21 ^a | nd ^a | 0.56 ± 0.32 ^a | nd ^a |
| <i>Rhus copallina</i> | Reference | nd ^a | nd ^a | nd ^a | nd ^a | nd ^a |
| | TNT | nd ^a | nd ^a | nd ^a | nd ^a | nd ^a |
| | RDY | 1.63 ± 0.21 ^b | 0.56 ± 0.18 ^a | nd ^a | nd ^a | nd ^a |
| | Comp B | 1.78 ± 0.24 ^b | nd ^a | nd ^a | nd ^a | nd ^a |

Table 4.4: Mean values with standard error for *U. alata* and *R. copallina* index values (n=3) across all treatments. Bold text indicates a significant difference from reference values and letter codes denote statistical grouping of values.

| | <i>Ulmus alata</i> | | | <i>Rhus copallina</i> | | |
|--|------------------------------|---------------------------------|------------------------------------|------------------------------|--------------------------------|------------------------------------|
| | Reference | RDX | TNT | Reference | Comp B | TNT |
| Carotenoid R1 | 0.0007 ± 0.0001 ^a | 0.0011 ± 0.0004 ^{a, b} | 0.0019 ± 0.0001^b | 0.0008 ± 0.0001 ^a | 0.00012 ± 0.0002 ^a | 0.0018 ± 0.0001^b |
| 680_630 | 1.04 ± 0.05 ^a | 0.91 ± 0.02 ^{a, b} | 0.81 ± 0.03^b | 0.99 ± 0.02 ^a | 0.92 ± 0.05 ^a | 0.84 ± 0.03 |
| 740_630 | 2.77 ± 0.20 ^a | 4.00 ± 1.35 ^{a, b} | 6.51 ± 0.22^b | 3.18 ± 0.07 ^a | 4.34 ± 0.83 ^a | 5.35 ± 0.25 ^a |
| 740_685 | 2.73 ± 0.30 ^a | 4.37 ± 1.71 ^{a, b} | 7.87 ± 0.35^b | 3.24 ± 0.14 ^a | 4.78 ± 1.08 ^{a, b} | 6.34 ± 0.45^b |
| 750_710 | 1.50 ± 0.04 ^a | 1.69 ± 0.06 ^{a, b} | 1.87 ± 0.03^b | 1.58 ± 0.02 ^a | 1.70 ± 0.10 ^a | 1.81 ± 0.01 |
| Greenness Index | 0.87 ± 0.09 ^a | 1.16 ± 0.09 ^{a, b} | 1.51 ± 0.09^b | 0.94 ± 0.05 ^a | 1.21 ± 0.17 ^a | 1.44 ± 0.10 ^a |
| Vogelmann Red Edge Index 1 | 1.25 ± 0.02 ^a | 1.33 ± 0.02^b | 1.39 ± 0.01^b | 1.28 ± 0.01 ^a | 1.33 ± 0.04 ^a | 1.37 ± 0.01 ^a |
| Photochemical reflectance index | -0.08 ± 0.002 ^a | -0.09 ± 0.007 ^a | -0.09 ± 0.005 ^a | -0.09 ± 0.005 ^a | -0.08 ± 0.001 ^a | -0.09 ± 0.003 ^a |
| Normalized Difference Veg Index | 0.51 ± 0.04 ^a | 0.67 ± 0.04^b | 0.79 ± 0.02^b | 0.58 ± 0.01 ^a | 0.67 ± 0.08 ^a | 0.76 ± 0.01 ^a |
| Plant Senescence Reflectance Index | 0.19 ± 0.03 ^a | 0.09 ± 0.02^b | 0.05 ± 0.01^b | 0.14 ± 0.01 ^a | 0.09 ± 0.04 ^a | 0.06 ± 0.01 ^a |
| Structure-Insensitive Pigment Index | 0.66 ± 0.02 ^a | 0.76 ± 0.03^b | 0.83 ± 0.02^b | 0.70 ± 0.01 ^a | 0.76 ± 0.05 ^a | 0.81 ± 0.01 ^a |
| Moisture Stress Index | 1.04 ± 0.09 ^a | 0.92 ± 0.08 ^a | 0.72 ± 0.13 ^a | 1.13 ± 0.03 ^a | 0.90 ± 0.09 ^{a, b} | 0.68 ± 0.05^b |
| Summation of Green Reflectance | 685.69 ± 31.14 ^a | 564.43 ± 111.16 ^a | 452.16 ± 40.28 ^a | 669.12 ± 18.91 ^a | 518.16 ± 46.35 ^{a, b} | 410.89 ± 35.68^b |
| Water Band Index | 0.96 ± 0.019 ^a | 0.97 ± 0.026 ^a | 0.99 ± 0.028 ^a | 0.96 ± 0.003 ^a | 0.95 ± 0.016 ^a | 1.02 ± 0.003^b |

Figure Legends

Figure 4.1: Map of field site highlighting Edgefield County in South Carolina USA. Inset map shows plot locations on field site with colored symbols identifying the contaminant present. Red is reference, Yellow is TNT, Green is Composition B, and Orange is RDX.

Figure 4.2: Light curves showing changes in electron transport rate (ETR) as photosynthetically active radiation (PAR) increases for *U. alata* (A) and *R. copallina* (B) individuals across treatment groups. Errors bars represent standard error.

Figure 4.3: Mean spectral profiles from hyperspectral image pixels across all treatment groups for (A) *U. alata* individuals and (B) *R. copallina* individuals.

Figure 4.4: Principal components analysis (PCA) ordination of spectral reflectance values for (A) *U. alata* individuals and (B) *R. copallina* individuals across treatments. Red lines indicate separation across axes.

Figure 4.5: Principal components analysis (PCA) ordination of hyperspectral index values for (A) *U. alata* individuals and (B) *R. copallina* individuals across treatments. Red lines indicate separation across axes.

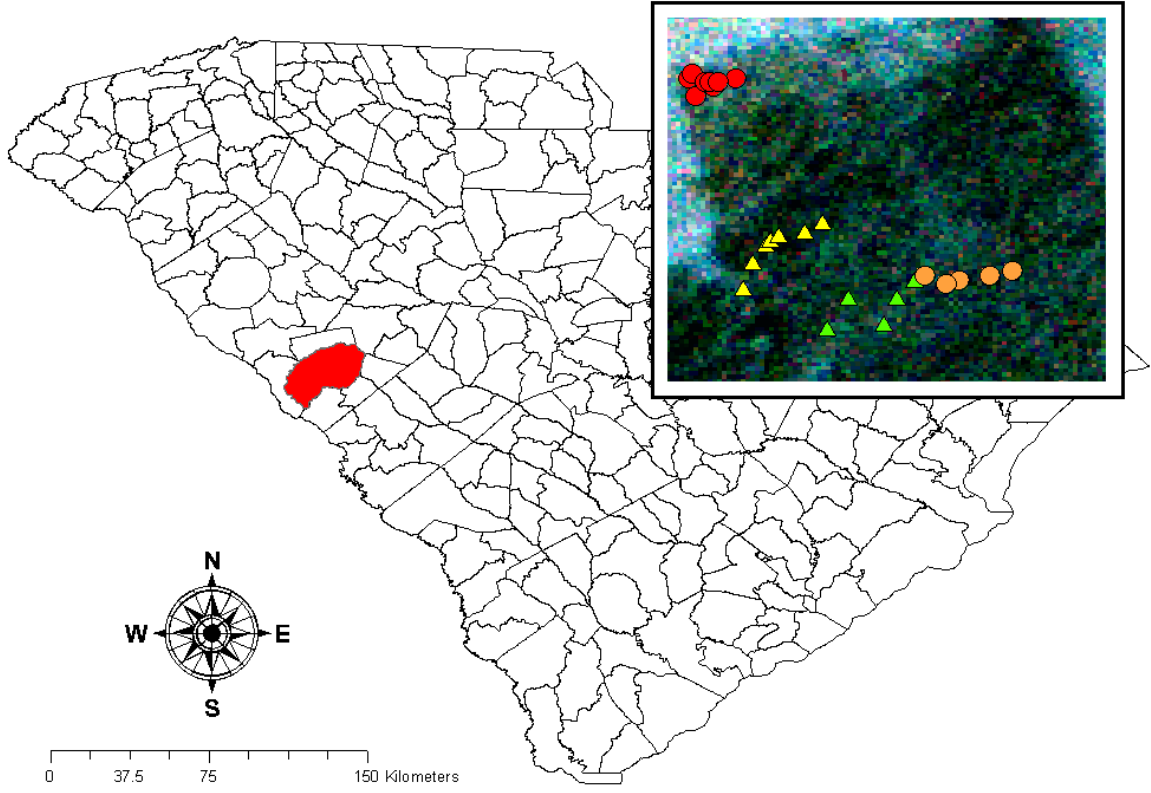


Figure 4.1

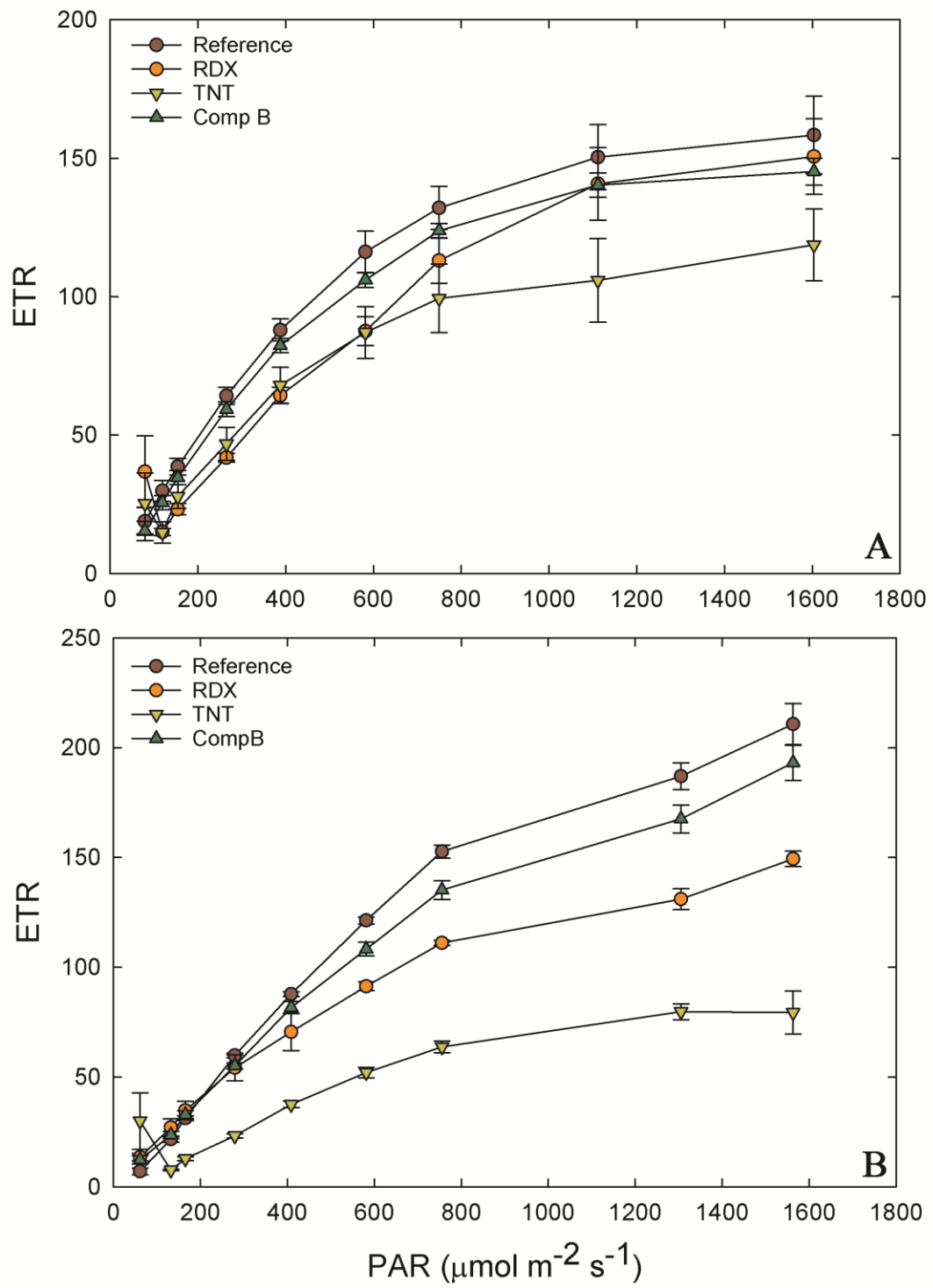


Figure 4.2

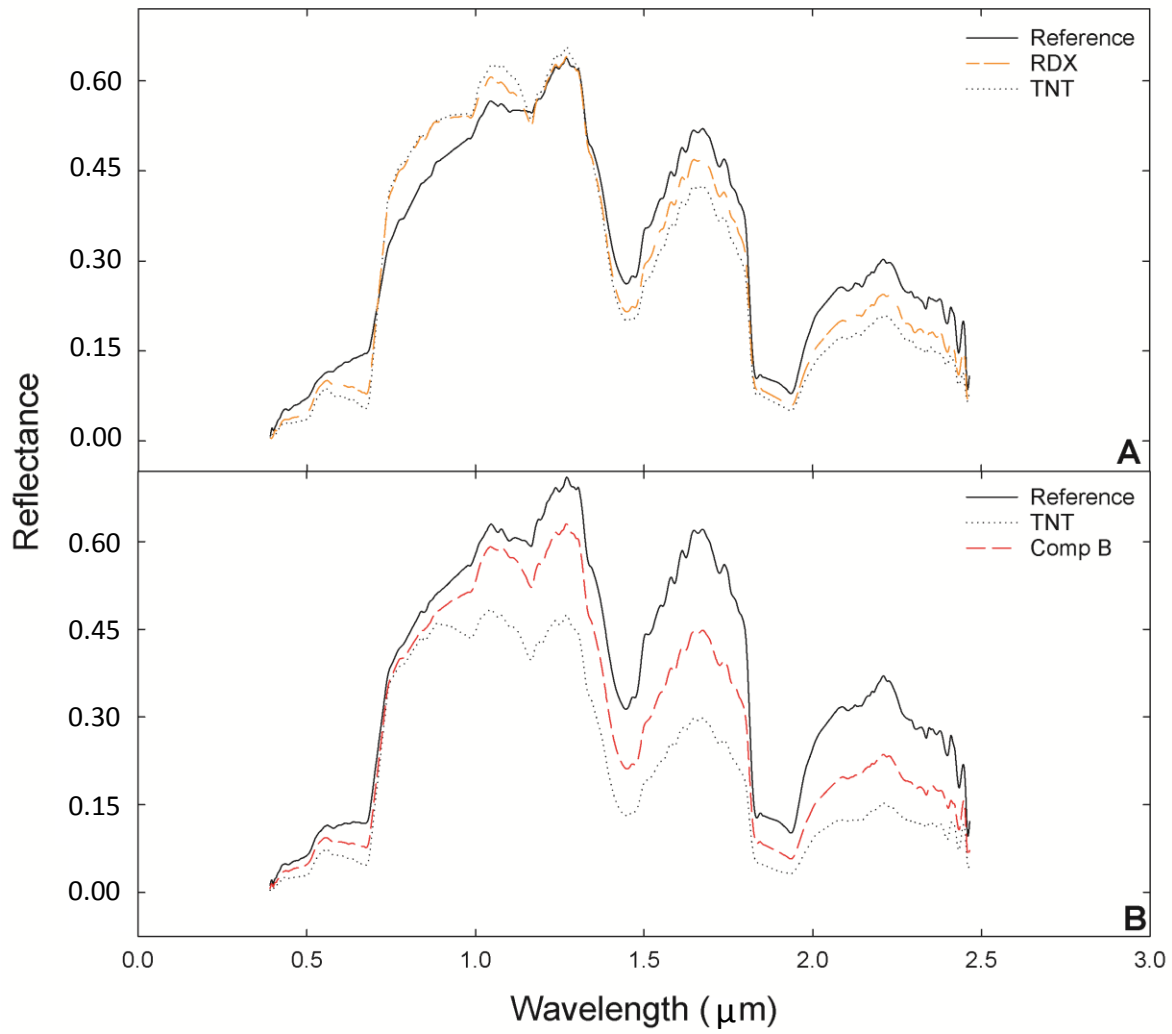


Figure 4.3

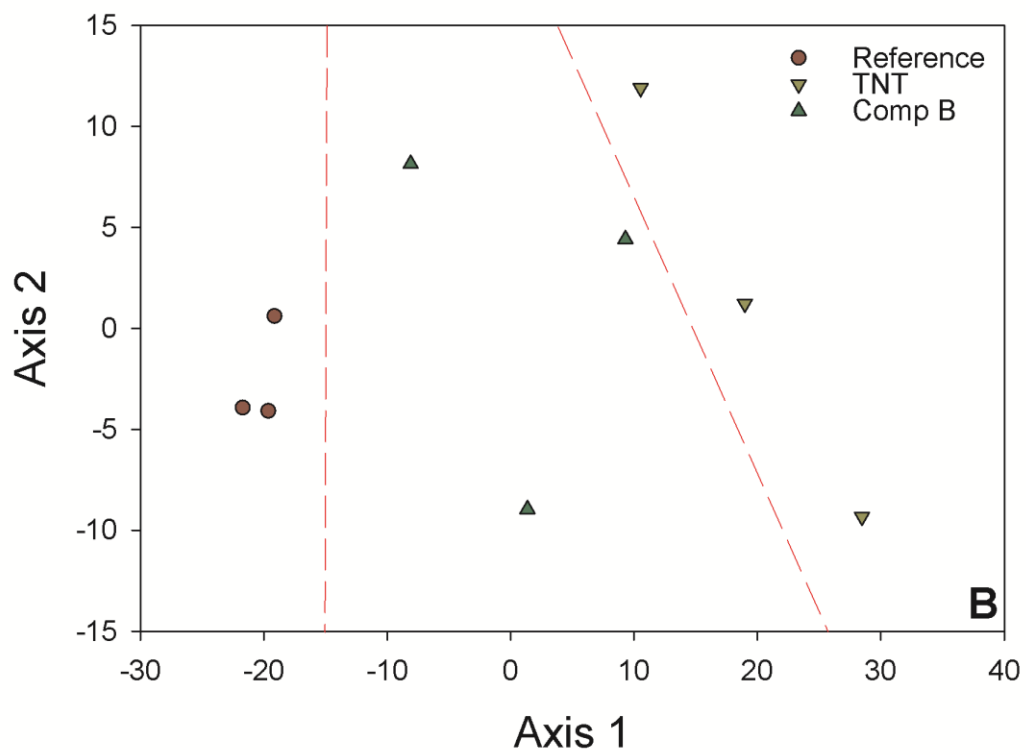
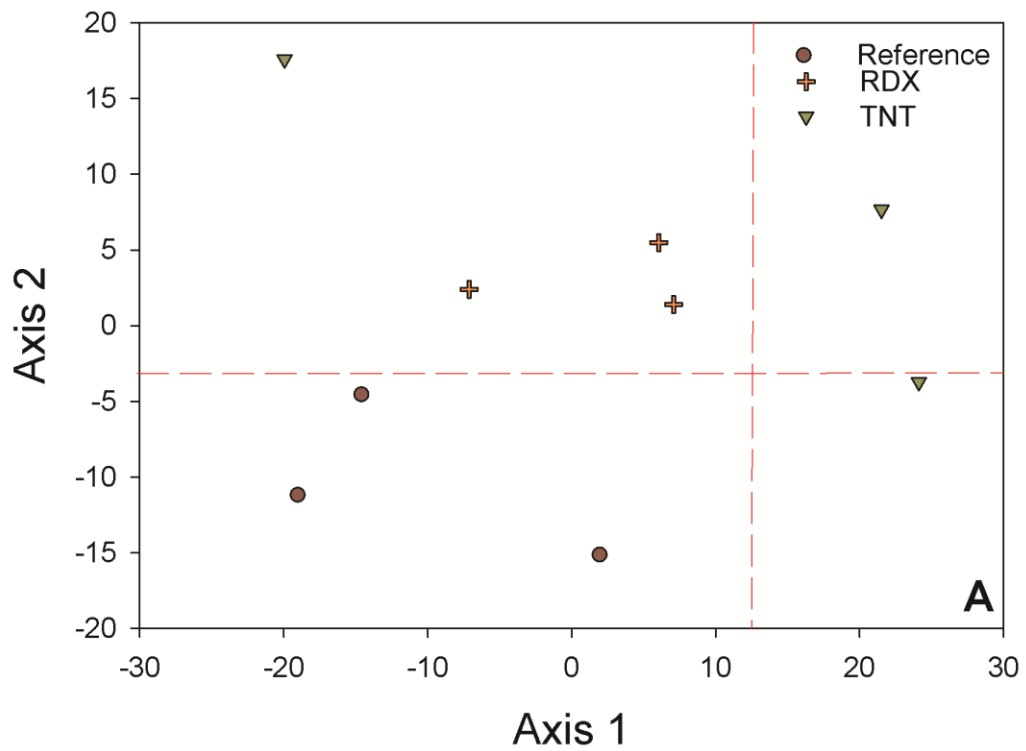


Figure 4.4

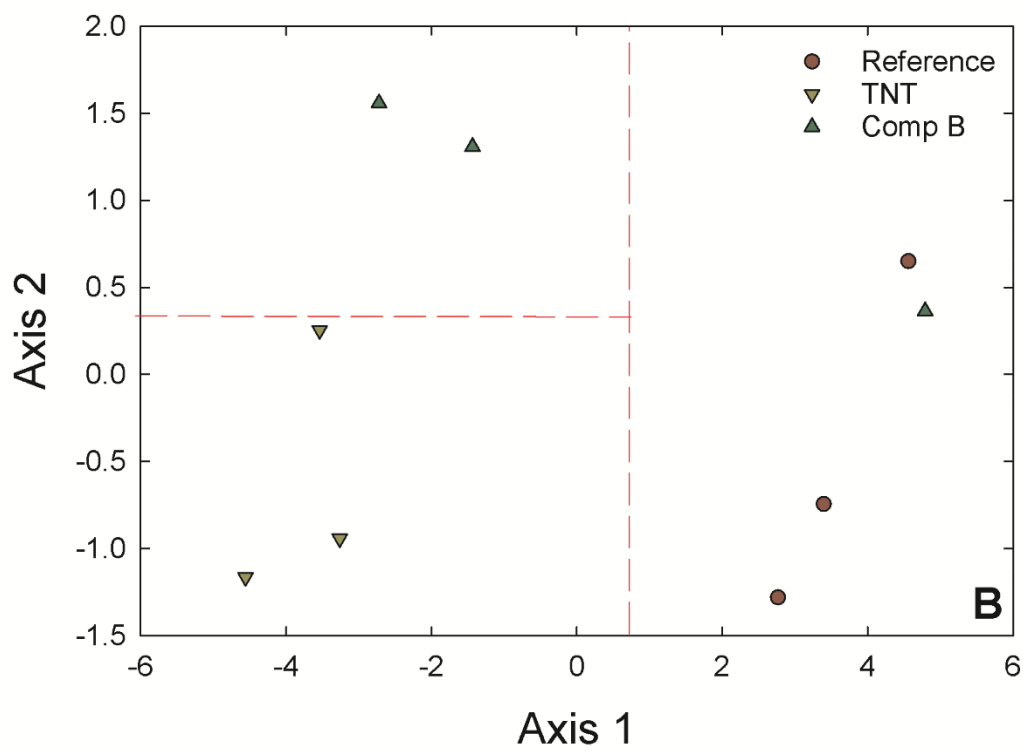
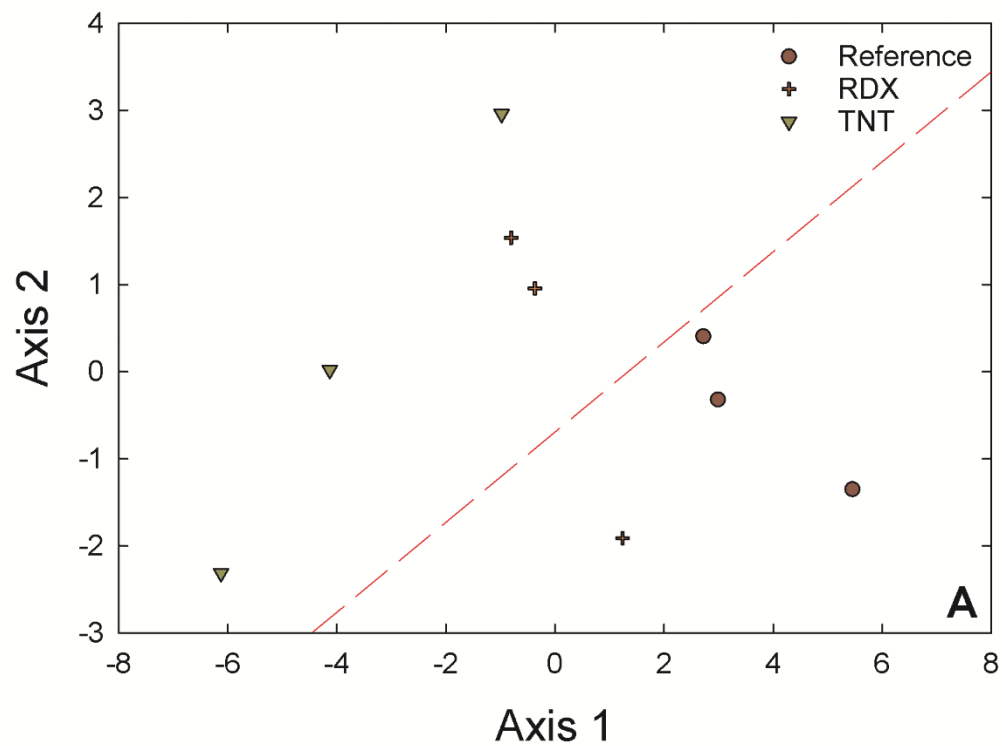


Figure 4.5

Summary

Species level physiological and spectral responses were clearly observable in the presence of explosive compounds both in the lab and in the field. Impacts of contaminant presence on vegetation health can directly influence vegetative community composition. Both species and functional composition were altered in the presence of explosives, however richness and diversity saw no significant changes. This is likely due to the establishment of more tolerant species filling in open areas left by extirpated sensitive ones. Explosive impacts were not only observable via ground-based measurements but also via aurally obtained hyperspectral data. This work has not only provided cross-scale linkages of explosive contamination responses for vegetation but also proposed using available remote detection technologies for the use of explosives detection.

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Appendix 1

Table A1: Herbaceous Species Percent Cover and Frequency for reference and explosives contaminated plots.

| Species | Percent Cover | | | | | | | | Frequency | | | |
|---------------------------------|---------------|-----|------|------|------|-----|--------|------|-----------|-----|-----|--------|
| | Reference | SE | RDX | SE | TNT | SE | Comp B | SE | Reference | RDX | TNT | Comp B |
| <i>Asteraceae sp.</i> | 0.9 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20 | 0 | 0 | 0 |
| <i>Rubus fruticosus</i> | 1.8 | 0.6 | 1.6 | 0.6 | 2.8 | 0.6 | 2.2 | 1.5 | 60 | 67 | 67 | 38 |
| <i>Juncus tenuis</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.2 | 0 | 0 | 0 | 13 |
| <i>Eupatorium capillifolium</i> | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 33 | 0 |
| <i>Adiantum pedatum</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 10 | 0 | 0 | 13 |
| <i>Vitis labrusca</i> | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 0 | 0 |
| <i>Panicum capillare</i> | 24.5 | 9.7 | 47.9 | 10.4 | 29.3 | 7.7 | 16.9 | 10.4 | 50 | 67 | 78 | 75 |
| <i>Poaceae</i> | 0.0 | 0.0 | 7.9 | 6.9 | 0.0 | 1.3 | 2.6 | 0.3 | 0 | 44 | 33 | 0 |
| <i>Cynodon dactylon</i> | 17.6 | 9.5 | 0.0 | 0.0 | 16.9 | 6.7 | 11.5 | 11.1 | 50 | 44 | 0 | 25 |
| <i>Grass 4</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.9 | 3.9 | 4.4 | 0 | 11 | 0 | 0 |
| <i>Lonicera japonica</i> | 15.9 | 6.8 | 0.4 | 0.4 | 13.9 | 0.1 | 0.1 | 10.4 | 80 | 11 | 11 | 75 |
| <i>Oenantha biennis</i> | 15.7 | 7.4 | 13.7 | 8.4 | 10.9 | 2.2 | 6.6 | 5.3 | 100 | 89 | 100 | 75 |
| <i>Solanum carolinense</i> | 0.7 | 0.4 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 40 | 0 | 22 | 13 |
| <i>Cyperus esculentus</i> | 0.3 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20 | 0 | 0 | 0 |
| <i>Oxalis stricta</i> | 0.4 | 0.3 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 20 | 22 | 0 | 13 |
| <i>Toxicodendron radicans</i> | 0.2 | 0.2 | 0.8 | 0.7 | 0.2 | 0.0 | 0.0 | 0.2 | 10 | 0 | 33 | 13 |
| <i>Ambrosia artemisiifolia</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 11 | 0 |
| <i>Smilax rotundifolia</i> | 0.2 | 0.2 | 0.4 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 11 | 0 |
| <i>Campsis radicans</i> | 1.5 | 0.6 | 2.1 | 0.6 | 1.9 | 0.6 | 1.4 | 0.6 | 60 | 56 | 78 | 75 |
| <i>Dicanthylom sp.</i> | 2.9 | 2.0 | 2.2 | 0.9 | 2.3 | 6.0 | 13.8 | 1.2 | 30 | 89 | 56 | 50 |
| <i>Lythrum salicarin</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 0.5 | 0.0 | 0 | 11 | 0 | 0 |
| <i>Passiflora incarnata</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 10 | 0 | 0 | 13 |
| <i>Galium aparine</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 0 | 0 |
| <i>Lespedeza cuneata</i> | 1.5 | 0.9 | 2.6 | 1.1 | 01.0 | 4.0 | 15.5 | 0.6 | 40 | 89 | 78 | 50 |
| <i>Verbena hastata</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.4 | 0.7 | 0.1 | 10 | 44 | 0 | 0 |
| <i>Unknown 4</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0 | 0 | 0 | 13 |
| <i>Carex brunnescenes</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 0 | 0 |
| <i>Setaria glauca</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 0 | 0 |
| <i>Panicum virgatum</i> | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0 | 0 | 0 |

| | | | | | | | | | | | | |
|------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|
| <i>Gnaphalium purpureum</i> | 0.6 | 0.4 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 30 | 11 | 0 | 13 |
| <i>Rumex sp.</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.5 | 0.8 | 0.3 | 0 | 44 | 0 | 13 |
| <i>Parthenocissus quinquefolia</i> | 0.6 | 0.4 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 30 | 0 | 22 | 0 |

Vita

Stephen Michael Via was born on June 5th, 1988 in Newport News, Virginia to Michael A. and Crystal U. Via. Growing up in a family of avid gardeners, Stephen was imbued early on with respect and appreciation for plants of all kinds. Spending his summers on road trips to the beaches of North Carolina and the mountains of Virginia with his family, Stephen was never far from a field guide and became proficient in identifying species of all forms of biota. He graduated from Heritage High School (Newport News, Va.) in 2006 and went on to receive his bachelor's in Biology from Virginia Tech (Blacksburg, Va.) in 2010. While at Virginia Tech he worked in several research laboratories as an undergraduate research assistant. Stephen then proceeded to obtain a Masters in Biology from Virginia Commonwealth University (Richmond, VA) while annoying his coworkers with his very Hokie centered wardrobe (good ole orange and maroon). Subsequently he enrolled in the Ph.D. program at VCU to continue his work with vegetation and explosives. It was then that he discovered that it was possible to wear gold and black at the same time and in some cases with a ram emblazoned on the shirt. As a side-effect of his Ph.D. work Stephen developed a near crippling affliction, commonly referenced to as "Orchid addiction". He now tries desperately to not kill the over 100 species of orchids he has obtained through this illness. Stephen will be leaving VCU and Richmond for a postdoctoral position at the Mt. Cuba Research center in Delaware, USA working on quantifying the status and ecology of native orchid populations.