Original article

Canopy gaps decrease microbial densities and disease risk for a shade-intolerant tree species

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A B S T R A C T

Canopy disturbances such as windthrow events have obvious impacts on forest structure and composition aboveground, but changes in soil microbial communities and the consequences of these changes are less understood. We characterized the densities of a soil-borne pathogenic oomycete (Pythium) and a common saprotrophic zygomycete (Mortierella) in nine pairs of forest gaps created by windthrows and adjacent forest understories. We determined the levels of Pythium necessary to cause disease by performing pathogenicity experiments using two Pythium species, a range of Pythium densities, and two common tree species (Acer rubrum and Prunus serotina) from the study sites. Three years post-disturbance, densities of Mortierella remained suppressed in soil from forest gaps compared to levels in intact forest understories while varying across sites and sampling dates. Pythium were infrequently detected likely because of soil handling effects. Expression of disease symptoms increased with increasing inoculum density for seedlings of P. serotina with each Pythium spp. having a similar effect on this species. Conversely, A. rubrum appeared resistant to the two species of Pythium. These results suggest that Pythium densities at sites where they were detected are sufficient to cause disease and possibly affect establishment of susceptible species like P. serotina. Because early seral environments have lower loads of the saprotrophic Mortierella, pathogen loads may follow a similar pattern, causing susceptible species to establish more frequently in those habitats than in late-seral forests. Forest disturbances that alter the disease landscape may provide an additional mechanism for explaining succession of temperate forests in addition to the shade-tolerance paradigm.

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1. Introduction

Disturbances are important processes affecting forest community composition (e.g. Peterson and Pickett, 1995) and resource availability. For example, forest gaps are known to affect light penetration to the understory (Ritter et al., 2005), soil moisture (Ritter et al., 2005), and fine root biomass (Wilczynski and Pickett, 1993). Gaps also alter trophic interactions. For example, aboveground herbivory varies in gaps vs. outside of gaps and in some cases counters the beneficial effects of increased growth in gaps (Tripler et al., 2005; Krueger et al., 2009). It is less clear how forest disturbances alter soil microbial communities and the consequences of these changes.

Soil-borne pathogens are known to alter the growth and survival of seedlings and saplings of both temperate (e.g. Packer and Clay, 2000, 2002; Romero et al., 2007; Reinhart and Clay, 2009) and tropical tree species (Augsburger, 1984; Hood et al., 2004; Augspurger and Wilkinson, 2007). Gaps in tropical forests appear to function as recruitment sanctuaries and may provide an escape from the effects of soil-borne pathogens (Fig. 1). Gaps could affect disease dynamics in complex ways that influence both the host and pathogen. For example, the increased growth rate of plants in gaps vs. understories may allow them to shorten the period of time they are most susceptible to disease (i.e. seedling developmental stage). Individuals that rapidly transition from seedlings to juveniles are likely to experience less disease than plants spending more time as seedlings either because these later stages of development contain more lignified roots and/or have more structurally complex root architecture to compensate for the loss of root segments to disease (Augsburger, 1990).

Gaps may also alter pathogen populations either directly or indirectly. Gap environments are drier (and warmer) than understory...
environments (Ritter et al., 2005) which may negatively affect the plants but also has a negative effect on some pathogen populations since their density and the expression of disease symptoms are often positively correlated with soil moisture (e.g., Augspurger, 1990; Erwin and Ribeiro, 1996; van Os and van Ginkel, 2001). Gaps may also be associated with changes in other soil microbial assemblages (e.g., mycorrhizal fungi) that have the potential to suppress development of disease by affecting the pathogens directly (e.g., competing for carbon and colonization space) or by affecting host plants (e.g., increased growth rates and phosphorus) (Borowicz, 2001).

A key question to be resolved is whether forest disturbances alter pathogen pressure and affect tree recruitment. A recent study reported that shade-tolerance rankings of seedlings of 21 tropical tree species were negatively correlated with their susceptibility to soil-borne diseases (McCarthy-Neumann and Kobe, 2008) but see another study on seed diseases, O’Hanlon-Manners and Kotanen (2006) included three congeneric (or closely related) pairs of shade-tolerant and shade-intolerant taxa. They found that seed diseases did not vary between gaps and understories and that only in one of three congeneric pairs did the shade-intolerant species experience greater disease than the shade-tolerant species. Others have suggested that related variation in survival and recruitment patterns used to test the Janzen—Connell hypothesis, potentially related to the effects of soil-borne disease (e.g., Packer and Clay, 2000), are not typical in temperate systems (Hyatt et al., 2003). Thus, further research in temperate forests is necessary to determine if there are general patterns and to specifically determine if habitats vary in the density of key soil pathogens, if this variation is ecologically significant (i.e., affects disease dynamics), and how potential host species vary in susceptibility.

In this study we estimated the density of a common soil-borne pathogenic oomycete (Pythium) and a common saprophytic zygomycete (Mortierella) in paired plots of forest windthrows (i.e., gaps) vs. adjacent intact forests in the Allegheny Plateau. Pythium species often have a wide host range, can severely reduce plant fitness, and can survive as saprophytes in the soil (Burdon, 1987; Jarosz and Davelos, 1995). Although much is known about Pythium densities and their density-dependent disease dynamics in agricultural systems (e.g., Burdon and Chilvers, 1975; Mitchell, 1975; Ingram and Cook, 1990), considerably less is known about their densities in natural plant communities and the relationship between their density, how it varies with habitat, and how density affects disease. We quantified the effects of a range of densities of two Pythium species on seedling performance of two dominant temperate tree species (Morin et al., 2006) that are known to differ greatly in their shade tolerance: Prunus serotina (shade intolerant) and Acer rubrum (shade tolerant) (Burns and Honkala, 1990). In line with results from other studies, we predict that pathogen density will be greatest in intact forests (e.g., Augspurger, 1984) and that the shade-intolerant species (P. serotina) will be most susceptible to disease (McCarthy-Neumann and Kobe, 2008).

2. Materials and methods

2.1. Study system and soil sampling

A severe thunderstorm created >200 forest windthrow gaps across the Allegheny Plateau region of northwestern Pennsylvania, USA in Jul-2003 (Evans et al., 2007). Alejandro Royo and colleagues identified 17 sites with pairs of gaps and intact forest for a series of studies. From these 17 sites, we randomly selected nine sites for our study. Each of the selected sites contained a forest gap ranging in size from 0.1 to 4 ha with mean of 1.26 ± 0.47 ha (±1SE) and a mean reduction in standing trees of 54.6 ± 5.35% (see panoramas in the Online Resource for examples of sampled windthrows). P. serotina and A. rubrum are two dominant tree species in this region (Morin et al., 2006).

We collected soil samples from the nine replicate pairs of forest gaps and adjacent intact forests in 2006, three years post-disturbance, during 19–20 Jun 2006 and 20–21 Aug 2006 (e.g., 3 composite soil samples × 2 habitat types [gaps vs. intact forest] × 9 sites × 2 sampling dates). During the June sampling period, one nearby gap was mistakenly sampled instead of the intended gap (gap reference 850 instead of 850). At each site and date, we collected three composite soil samples inside and outside each gap. Each composite sample consisted of five haphazardly selected soil cores (2.2 cm diameter core and 0 to −10 cm sampling depth). A total of 108 soil samples were collected during the two sampling periods. Each composite sample was homogenized and air dried for 1 week and stored at room temperature.
2.2. Microbial density

We quantified the density of Pythium in soil using the plate dilution-frequency technique (Lumsden et al., 1975) during June–2007. Concurrent work on another study also isolating Pythium from forest soils revealed the need to refine our methods to better differentiate Pythium from Mortierella, which occurred at greater densities and inflated initial estimates of Pythium (unpublished results). After refining our technique, we were able to differentiate the two genera but by now the air-dried soils had been stored for ca. a year. Logistically it was not possible to recollect samples because some of the sites had undergone timber harvests soon after the initial collections. Thus, the reported estimates of Pythium density are likely to be conservative because of the length of time the soils were stored (Aderungboye, 1976). However, Pythium propagules can persist in air-dried soil for many years (Hoppe, 1959). Samples of air-dried soil (ca. 2 g) were added to 30 ml of diluted agar (henceforth referred to as water agar; i.e. 3 g of Difco Bacto agar per liter of deionized water) and mixed on a shaker for 10 min. After mixing, a serial dilution was performed using test tubes filled with dilute water agar. Plates were established according to Lumsden et al. (1975) on selective growth media (P5ARP) (Martin, 1992). After incubation in the dark for 24 h at 25 °C, the number of droplets with Pythium per dilution step were counted. If plates were incubated longer, then the slower growing but more prevalent Mortierella would inflate frequency counts. The total number of droplets in the series with Pythium were converted into the number of propagules per gram of air-dried soil by reference to a standard table (Harris and Sommers, 1968). Eight isolates were selected at random and maintained on cornmeal agar. Later they were identified and a subset used in the pathogenicity experiments.

We also quantified the density of Mortierella, a common zygomycete, to determine whether forest disturbances disrupt other soil microbial taxa. We estimated their density in soil samples by dilution plating and colony counts during autumn of 2006. Samples of soil (ca. 10 g) were added to 50 ml of dilute agar (2 g of Difco Bacto agar per liter of deionized water) and mixed on a shaker for 10 min. After mixing, a serial dilution was performed using test tubes filled with another agar solution (3 g of Difco Bacto agar per liter of deionized water). A small volume of soil solution was then spread over five replicate PAR5P plates per dilution step. Plates were incubated for 3 days in the dark at 25 °C. Mortierella density estimates were averaged across the five plates and included Mortierella and Pythium. We refer to these as Mortierella densities because Pythium occurred at densities that were orders of magnitude less than Mortierella (unpublished data and other studies [see Fig. 2 here vs. data from Reinhart and Clay, 2009]).

We tested the effect of habitat type (gap vs. intact forest) and sampling date on Pythium density using ANOVA with Proc Mixed in SAS version 9.13 (SAS Institute Inc., CaryNC, USA). Proc Means was used to collapse the three density estimates per experimental unit per sampling date (i.e. habitat type × date × site) into a mean. The averaged density data were rank transformed. Habitat type (i.e. gap) and sampling date were fixed effects and gap/site, and gap × date/site were treated as random effects. This analysis was repeated for Mortierella density data; however, those data were log transformed.

2.3. Effect of Pythium species and density

We tested the effect of a range of densities of two Pythium species on seedling performance (defined below) using a full factorial experimental design to determine if increasing inoculum density increased disease expression and whether a density threshold was present that could be related to the observed densities in the field reported in this study and others (Reinhart and Clay, 2009). We selected Pythium sylvaticum and an unknown Pythium sp. isolated from our soils for use in the pathogenicity experiment. Isolates were identified by Gloria Abad, molecular biologist/plant pathologist, USDA-APHIS Plant Safeguarding and Pest Identification, National Identification Services, Molecular Diagnostics Lab, Beltville, MD, USA.

Pathogen density treatments were established using soils containing known concentrations of Pythium species according to methods by Ingram and Cook (1990) to create experimental soils with different levels of Pythium infestation. In order to produce the inoculum for each Pythium spp. necessary to produce infested soil treatments, each species had to be grown in different media to obtain reproductive structures (i.e. oospores and sporangia). Py. sylvaticum was grown in liquid molasses and the unknown Pythium species in V-8 cultures (see Martin, 1992). Overall, we had trouble producing sufficient quantities of inocula (i.e. reproductive structures) of the unknown Pythium relative to Py. sylvaticum which were barely sufficient for the first experiment and affected the number of density levels in the second experiment.

We used separate experiments to evaluate responses of P. serotina and A. rubrum to Pythium. Seed used in the two experiments originated from Pennsylvania and was purchased from a commercial seed supplier (Sheffield’s Seed Co., Inc. Locke, NY, USA). Seed was surface sterilized prior to being cold stratified (i.e. soaked in solution of 10% bleach for 10 min and then thoroughly rinsed with RO water). The P. serotina experiment was...
performed in the spring of 2008. An infested soil was added to a sterilized (once autoclaved) 1 to 1 mix of potting soil and sand to obtain final densities of 0, 5, 15, 40, and 100 germinable propagules per gram of soil for the P. serotina experiment (n = 5 pots per Pythium species per inoculum density treatment) following Ingram and Cook (1990). These densities are well within the range of Pythium densities reported in other forested systems (Aderungboye, 1976; Reinhart and Clay, 2009). Pots for both experiments received 410 g of soil (50 total pots).

The A. rubrum experiment was performed in the spring of 2009. Infested soils remaining from the P. serotina pathogenicity experiment were stored at 4 °C until used to re-establish cultures for the second pathogenicity experiment. This required re-isolation of the Pythium onto agar plates and amplification in liquid cultures (described above). The A. rubrum experiment had Pythium densities of 0, 5, 15, 40, 100, and 250 germinable propagules per gram of soil (n = 5–6 pots per Pythium species per inoculum density treatment) (58 total pots). Because of insufficient inocula production, the density range for the unknown Pythium was constrained (0–40) for the A. rubrum portion.

Both experiments lasted 49 days. Pots were planted with three seedlings per pot and watered to field capacity. We placed clear plastic cups (946 mL) on top of each pot to maintain stable soil moisture conditions. Pots were maintained in a growth chamber at 20.5 °C with a 12 h light per day (PAR, ~180 μmol × m⁻² × sec⁻¹) and periodically watered and randomized. Humidifiers were added to increase humidity in the chambers. Every 3–4 days seedlings were checked to document the date of mortality and shoots (stems and leaves) were weighed at the conclusion of the experiment. After a period of time (28 d for P. serotina experiment and 18 d for the A. rubrum experiment), the plastic cups were removed then pots were watered as needed. Plant pathology trials often cover pots with clear cups to maintain a stable environment for the seedlings to grow and for the pathogen to interact with the seedlings. However, it appeared that disease expression (i.e. damping-off symptoms) was suppressed by the cups because the seedlings experienced limited water stress. For example, most of the P. serotina seedlings scored as dead (76%) did not die until after the cups were removed.

A composite variable of mortality and production data was used to test effects (sensu McCarthy-Neumann and Kobe, 2008):

\[
\text{Seedling performance per pot} = (\text{mean shoot biomass} \times \text{mean life span})/\text{days of experiment}^{-1}. 
\]

The mean shoot biomass was estimated only for the seedlings that were alive at the end of the experiment. We fit the P. serotina and A. rubrum data separately using mixed models with Proc Mixed in SAS version 9.13 (SAS Institute Inc., CaryNC, USA). We tested the effect of Pythium species and their initial density on seedling performance. Pythium species and inocula density were treated as fixed effects. Differences of Least Squares Means were also calculated using Proc Mixed (SAS Institute).

3. Results

3.1. Microbial density

*Mortierella* densities, which were two to three orders of magnitude greater than Pythium densities, were reduced by 32% in windthrown gaps vs. adjacent intact forest (ANOVA, \(F_{1,7} = 5.37, P = 0.054\)) (Fig. 2). *Mortierella* densities increased by 53% from Jun-2006 to Aug-2006 (\(F_{1,7} = 8.79, P = 0.021\)). Densities ranged from 5979 to 56,139 propagules per gram of soil in Jun-2006 and 12,279 to 64,936 in Aug-2006. There was no interaction between gap treatment and sampling date (\(F_{1,7} = 12.22, P = 0.31\)).

Pythium was not recovered among the samples collected in Jun-2006 and small amounts were detected (5 [range 0–38 propagules per gram of soil]) in soils during the Aug-2006 sampling period from the intact forest three years following windthrow (Fig. 3). There was no statistically significant effect of habitat type (ANOVA, \(F_{1,7} = 2.16, P = 0.19\)), sampling date (\(F_{1,7} = 2.16, P = 0.19\)), and no interaction between forest type and sampling date on Pythium densities (\(F_{1,7} = 2.16, P = 0.19\)). As mentioned in the Methods section, the density estimates are likely conservative because the duration of the soil storage which helps explain why Pythium was not detected at most of the sites.

3.2. Effect of Pythium species and density

Increasing the density of *Pythium sylvaticum* and *Pythium* sp. from zero to 100 propagules × gram of soil⁻¹ resulted in a 52% and 47% reduction in seedling performance of *P. serotina*, respectively (ANOVA, \(F_{4,37} = 4.17, P = 0.007\)) (Fig. 4). This combined with the density results above suggest that intact forest and sites with greater Pythium densities are likely to cause greater levels of disease for *P. serotina*. Both Pythium species behaved similarly (\(F_{1,7} = 1.84, P_{\text{species}} = 0.18\)) and no interactive effect between species and density was observed (\(F_{4,37} = 0.17, P_{\text{species}\times\text{density}} = 0.95\)). In contrast to *P. serotina*, increasing the density of the Pythium inocula had no effect on the performance of *A. rubrum* seedlings (ANOVA, \(F_{4,48} = 0.60, P = 0.70\)). There was no effect of *Pythium* species (\(F_{1,48} = 0.16, P = 0.70\)), and there was no interactive effect between species of *Pythium* and their densities (\(F_{4,48} = 0.04, P_{\text{species}\times\text{density}} = 0.99\)) on *A. rubrum* seedlings suggesting *A. rubrum* is relatively resistant to the two tested Pythium spp.

4. Discussion

4.1. Gap creation and soil microbial communities

Canopy disturbances such as windthrows can have large effects on forest structure and plant community composition (Peterson and Pickett, 1995). Gaps are known to alter a range of factors that affect soil microbial communities including decreases in fine root abundance (Wilczynski and Pickett, 1993; Ostertag, 1998), decreases in...
soil moisture and increases in soil temperature (Ritter et al., 2005). Soil pathogen activity has also been reported to increase in soil-borne pathogens (e.g. Aderungboye, 1976); however, Pythium propagules are known to persist in air-dried soil for many years (Hoppe, 1959). However, we documented Pythium in samples from only a few sites when they likely occur across all sites. In another study using identical methods, the authors reported Pythium densities in intact forest ranged from 5.1 ± 3.1 (mean ± 1SE) to 517.5 ± 75.2 propagules per gram of soil around P. serotina in more recently collected soils (overall mean = 332 propagules per gram of soil per tree) in a temperate deciduous forest in southern Indiana (Reinhart and Clay, 2009). Techniques that do not rely on culturing, such as quantitative PCR (qPCR), are likely to improve estimates of density but still face some challenges (Schoeder et al., 2006). Although our description of Pythium density across all sites is imperfect, they are suggestive and correspond with related generalizations that pathogenic activity is greater in intact forests than gaps (e.g. Fig. 1) and that an important abiotic regulator of Pythium populations (soil moisture) is lower in gaps than intact forest (e.g. Ritter et al., 2005).

4.2. Do gaps provide refuge from soil-borne pathogens?

Others have reported soil-borne pathogens can affect temperate tree establishment (Reinhart et al., 2005; Romero et al., 2007; Reinhart and Clay, 2009) but did not specifically compare the pathogenic activity of different habitat types (i.e. gap vs. intact forest). As mentioned previously, intact forests appear to be associated with greater loads of the saprotrophic Mortierella than gaps. This variation in density for Mortierella by habitat may typify patterns for other microbes including those that cause damping-off disease (i.e. Pythium). Our pathogenicity experiment confirmed that observed densities of Pythium at some of our study sites are sufficient to cause disease and may alter seedling recruitment patterns. Increasing Pythium density to a threshold of 20–100 Pythium propagules per gram of soil, similar to densities observed in intact forests, resulted in significant decreases in performance of P. serotina seedlings. However, Pythium had no effect on A. rubrum seedlings which appear resistant to the two Pythium spp. used in the pathogenicity trials (Fig. 4). Although Pythium spp. are often described as being generalist pathogens (e.g. Burdon, 1987), recent research suggests that Pythium spp. have intermediate levels of interaction specificity and can impact some, but not all, potential host species (Augspurger and Wilkinson, 2007). Furthermore, our data appear to suggest a saturating effect of inoculum density on P. serotina at approximately 40–100 propagules per gram of soil where further increases in pathogen density cause negligible increases in disease expression. Thus, our experiments suggest that the Pythium densities found in intact forests at some of our study sites and from other study sites (e.g. Reinhart and Clay, 2009) have the potential to inhibit seedling performance and establishment.

Greater light levels within our study gaps (Krueger et al., 2009) may have resulted in higher soil temperatures and lower soil moisture levels, which are generally unfavorable to soil microbes like Pythium (Augspurger, 1990; Martin and Loper, 1999; van Os and van Ginkel, 2001; Romero et al., 2007; Wielgoss et al., 2009). Thus, canopy gaps may decrease microbial densities below levels sufficient to cause disease for some tree species, thereby providing refugia for susceptible species (Fig. 1) (Augspurger, 1984; O’Hanlon-Manners and Kotanen, 2004).

4.3. Covariance of shade- and pathogen-tolerance?

The fundamental growth/survival tradeoff that allows tree species to either establish in gaps or persist under deeply shaded understories is a basic tenet of the shade-tolerance paradigm (e.g. Shugart, 1984; Pacala et al., 1994). Nevertheless, the suite of traits possessed by seedlings and saplings of shade-tolerant species (e.g. dense wood,
well defended leaves, greater carbohydrate storage) that allow greater survival under prolonged low-light conditions may simultaneously confer increased defense against protracted enemy pressure (Coley et al., 1985; Myers and Kitajima, 2007; McCarthy-Neumann and Kobe, 2008). Our results demonstrate the shade tolerance of the two dominant seedling species recruiting in forests within the Alleghe Rny Plateau region are positively correlated with pathogen-tolerance. The relatively shade-intolerant P. serotina was found to be more susceptible to damping-off disease caused by two Pythium species than the more shade-tolerant A. rubrum (Fig. 4). Other studies have similarly found that P. serotina is more susceptible to damping-off disease than other temperate tree species regardless of their shade-tolerance classification (Packer and Clay, 2000). Thus, survival in shaded forest understories may arise from the physiological ability to survive at low light, which may be correlated with traits that decrease susceptibility to natural enemies (e.g. herbivores, pathogens), or relate to both physiological constraints associated with shade and susceptibility to natural enemies. Apparent competition may also be a factor affecting successional dynamics if shade-tolerance classifications correspond with asymmetric effects of disease (e.g. Cobb et al., 2010). For example, if recovering forests are established by A. rubrum and it functions as a reservoir species and amplifies Pythium in the soil then this may negatively affect the abundance of susceptible species like P. serotina which likely share this group of promiscuous soil-borne pathogens. Although limited to two species, our data support the growing body of evidence from both temperate (O’Hanlon-Manners and Kotarán, 2004, 2006) and tropical forest systems (McCarthy-Neumann and Kobe, 2008) indicating a positive correlation between shade tolerance and disease resistance. Interestingly, A. rubrum and P. serotina seedlings had similar overall performance values (~0.09) under pathogen densities typically observed in undisturbed forests (Reinhart and Clay, 2009), whereas under the low pathogen loads, P. serotina’s performance was nearly double that of A. rubrum. This variation in performance between species with varying levels of pathogen pressure suggest that interactions with soil-borne pathogens may contribute to species co-existence across heterogeneous forests by promoting differential establishment patterns of these two dominant tree species across habitat types (gaps vs. intact forests).

5. Conclusions

Although this study demonstrates that forest disturbance alters components of the soil microbial community, which in turn may influence important ecological processes including disease, both the density estimates and pathogenicity experiments provide only glimpses of the complex processes occurring in the soil. Covarying changes in soil resources (nutrients and water), herbivory, competition, and other changes in soil microbial communities may also alter recruitment dynamics. However, results from this study and related studies suggest that changes in microbial density and host resistance to disease may have important consequences on disease prevalence and ultimately on tree recruitment and forest succession. To improve our understanding of succession following forest disturbance more research is needed on belowground microbial populations and their interactions with plants to resolve the importance of fluctuating enemy densities, enemy resistance, and plant physiological adaptations to shade.

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