

Nitrogen source preference of *Aspergillus sydowii*, an infective agent associated with aspergillosis of sea fan corals

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Abstract

We attempt to identify the mechanism by which nitrogen enrichment increases the prevalence of aspergillosis, a disease caused by the terrestrial fungus *Aspergillus sydowii*, which infects sea fan corals (*Gorgonia ventalina*) throughout the Caribbean, by looking at the metabolic capabilities of the pathogen to (1) determine whether *A. sydowii* can catabolize sea fan–derived nitrogen, (2) ascertain fungal preference for different nitrogen sources, and (3) determine whether fungi isolated from diseased sea fans preferentially assimilate sea fan–derived nitrogen. Stable nitrogen isotope experiments demonstrated that *A. sydowii* is capable of assimilating and prefers sea fan–derived nitrogen (72% of available nitrogen) to nitrate and more readily assimilates nitrogen from coral tissue than gorgonin skeleton. Variation in the proportion of sea fan–derived nitrogen assimilated by different fungal isolates was significant, with those from diseased sea fans showing greater nitrogen assimilation of sea fan tissue.

Worldwide, coral reefs are experiencing a period of decline. Wilkinson (2008) estimates that as of 2008, 20% of the world's coral reefs have been destroyed, and an additional 15% are in critical danger of collapse because of anthropogenic influences. Caribbean reefs have suffered the most; 80% of hard coral cover has been lost in the past three decades (Gardner et al. 2003; Wilkinson 2008). Concurrent with accelerating decline of reefs worldwide, the prevalence of coral disease has increased in the last 20 yr (Harvell et al. 1999; Bruno et al. 2003; Harvell et al. 2007), especially in the Caribbean, a known disease “hotspot” (Harvell et al. 1999). In addition to increased coral mortality and reduced reproduction and growth, disease can cause loss of coral community structure and diversity (Loya et al. 2001).

There is a dearth of data on factors influencing marine diseases, despite increasing observations of outbreaks (Kim and Harvell 2004; Lafferty et al. 2004). Factors contributing to the effects of marine disease include climate warming, pollution, nutrient enrichment, sedimentation, overharvesting of marine organisms, and introduction of pathogenic and infective agents (Harvell et al. 2004; Lafferty et al. 2004; Bruno et al. 2007), many of which have increased concurrently with disease prevalence (Williams and Bunkley-Williams 1990). Despite the numerous documented correlations between disease outbreaks and environmental stressors, the specific mechanisms of action are poorly understood.

One coral disease with strong connections to anthropogenic influences is aspergillosis, a fungal infection of gorgonian corals, including the common sea fan, *Gorgonia ventalina*. Abundant on Caribbean reefs and an integral member of the reef ecosystem, *G. ventalina* provides refuge

for fish (Bayer 1961) and can be found in densities of up to 1 sea fan m⁻² (Toledo-Hernandez et al. 2007). Aspergillosis was first documented near Saba, Netherland Antilles, in 1995 (Nagelkerken et al. 1997a), and within 1 yr, up to 90% of sea fans Caribbean-wide were infected (Nagelkerken et al. 1997b). Caused by the terrestrial fungus *Aspergillus sydowii* (Smith et al. 1996; Geiser et al. 1998), signs of aspergillosis include galls and lesions that spread throughout the sea fan, killing the tissue and exposing the gorgonin skeleton. Halos of purple tissue surrounding the lesions are the result of an inflammatory immune response by the coral (Ellner et al. 2007; Mydlarz et al. 2008). On pristine reefs, constitutive levels of sea fan immune defenses are sufficient to prevent infection by *A. sydowii* (Dube et al. 2002), but environmental stressors can weaken coral immune systems (Mydlarz et al. 2008), allowing the fungus to invade the sea fan skeleton. Histological evidence suggests that the infection spreads through the coral as the fungus grows within the gorgonin skeleton (Ellner et al. 2007). Studies examining the dynamics of the fungus–coral pathosystem assume that *A. sydowii* directly consumes sea fan tissue (Ellner et al. 2007), but this has never been demonstrated experimentally. Our knowledge about the ecological drivers of aspergillosis, especially with regard to temperature and nutrients (Baker et al. 2007; Bruno et al. 2003; Ward et al. 2007), and our ability to culture the pathogen in the laboratory, make this system a valuable model for understanding mechanisms of infection and the role of environmental drivers in coral disease.

Nutrient enrichment, defined for our purposes as conditions when concentrations of nitrogen and phosphorus are sustained above their long-term averages (Dubinsky and Stambler 1996), is a global, worsening problem in coastal waters (Lotze et al. 2006). Most commonly caused by the introduction of sewage and agricultural fertilizers into coastal marine ecosystems (Dubinsky and Stambler 1996), nutrient enrichment is hypothesized to increase the growth of marine fungi due to a release from nitrogen

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limitation (Olutiola and Cole 1977). However, this has not been assessed for most species, including *A. sydowii*. In terrestrial plants, the prevalence and severity of disease caused by fungal phytopathogens is known to increase with release from nitrogen limitation (reviewed by Snoeijs et al. 2000; Solomon et al. 2003). Along with an increase in the amount of nitrogen, coastal nutrient enrichment also changes the forms of nitrogen available. *A. sydowii* may utilize inorganic nutrients suspended in the water column or assimilate organic nutrients from the sea fan's gorgonin skeleton, coenenchyme, polyps, or zooxanthellae (the latter three components henceforth collectively referred to as "tissue"). In typical oligotrophic conditions found on coral reefs, the concentration of nitrogen in the water column is very small compared with the pool of organic nitrogen within the coral host. However, increased inorganic nitrogen in the water column offers a bioavailable source to the fungus. In eutrophic waters, fungi can avoid the energetic costs of metabolizing sea fan-derived nutrients (via increased enzyme production, etc.) by direct uptake of nutrients from the water column.

Many studies have linked increased nutrient inputs to altered coral community structure and diversity on reefs (Szmant and Forrester 1996), as well as to increases in coral disease severity and prevalence. Bruno et al. (2003) found that increased nutrient concentrations significantly increased disease severity in two Caribbean syndromes: aspergillosis and yellow band disease. Similar fertilization experiments caused a doubling in the rate and magnitude of tissue loss from black band disease in the reef-building coral *Siderastrea siderea* (Voss and Richardson 2006). The nutrients and bacteria in sewage have also been correlated with increases in coral disease (Patterson et al. 2002; Kaczmarek et al. 2005). The prevalence of aspergillosis is positively correlated with long-term averages of total nitrogen (TN) concentration in the Florida Keys, suggesting that nitrogen availability might influence the dynamics of this disease (Baker et al. 2007). TN is a measure of all forms of dissolved and particulate organic and inorganic nitrogen. One possible mechanism of this facilitation is increased pathogen growth and virulence through a general reduction of nutrient limitation (Kim and Harvell 2002) or an increase in the availability of preferred nitrogen sources. Alternatively, increased nitrogen concentrations could alter the physiology of the host, its zooxanthellae or associated bacteria, resulting in reduced sea fan immune function; however, this was not a focus of our study. Here we examine fungal preferences for nitrogen sources readily available in nutrient-enriched conditions as a first step in investigating the mechanisms by which nitrogen enrichment affects the prevalence and severity of aspergillosis.

The purpose of this study was threefold. (1) We used stable isotope analyses to determine whether *A. sydowii* is capable of assimilating sea fan-derived nitrogen and to ascertain whether the fungus preferentially assimilates nitrogen from sea fans or nitrate. We hypothesized that *A. sydowii* can assimilate sea fan-derived nitrogen but prefers ambient nitrate when both sources are present because of energetic efficiencies. (2) We examined nitrogen source preference in detail using stable isotope analyses to

determine fungal preference for sea fan gorgonin skeleton and sea fan tissue. We chose these two components because histological preparations reveal that *A. sydowii* is most commonly found in the gorgonin skeleton of infected sea fans, although the fungal pathogen must penetrate through the more easily catabolized sea fan tissue to reach the skeleton (Ellner et al. 2007). Furthermore, infected colonies often have damaged or dying tissues, but the skeleton will persist even after complete tissue loss. The gorgonin skeleton is approximately 11% N and 36% C, whereas sea fan tissue is about 2.5% N and 19% C (data not shown). Given these observations, as well as the known durability of gorgonin and the stability of its amino acid composition over time (Goldberg 1978; Sherwood et al. 2005), we hypothesized that if *A. sydowii* can catabolize sea fans, it will be more likely to use sea fan tissue as a nitrogen source. (3) The aforementioned experiments were conducted with five isolates of *A. sydowii* collected from a variety of substrates to test the hypothesis that fungi cultured from diseased sea fans are better at assimilating sea fan-derived nitrogen.

Methods

Experiment 1. Sea fan nitrogen assimilation: Organic vs. inorganic nitrogen preference—To determine whether *A. sydowii* can assimilate sea fan-derived nitrogen and to determine its preference for sea fan vs. inorganic nitrogen, the fungus was grown on agar media containing 40.0% glucose, amended with one of three nitrogen source treatments: nitrate alone (0.13 mol N L⁻¹; 0.0108 g nitrate mL⁻¹), homogenized sea fan gorgonin skeleton and tissue (henceforth referred to as homogenized sea fan, HSF) alone (0.13 mol N L⁻¹; 0.0255 g HSF mL⁻¹), or HSF + nitrate (0.064 mol N L⁻¹ each; 0.00542 g nitrate mL⁻¹ and 0.0127 g HSF mL⁻¹). Each treatment was mass balanced for nitrogen by source. The HSF comprised dried sea fan tissue and gorgonin skeleton from samples of healthy *G. ventalina* collected off the coast of Florida and homogenized in a SPEX CertiPrep cryogrinder with the use of liquid nitrogen. In the HSF alone and HSF + nitrate treatments, the plates contained two sources of carbon: the glucose in the minimal media and sea fan-derived carbon.

Five isolates of *A. sydowii* were used, with five replicates of each isolate per treatment. Isolates AS16 (FK11), AS17 (SS7), and AS18 (SA25) were cultured from diseased *G. ventalina* collected in the Florida Keys, the Bahamas, and the Netherland Antilles, respectively (Smith et al. 1996; Geiser et al. 1998). AS35 was cultured from a human clinical sample (NRRL 254), and AS72 was cultured from mangroves (NRRL 247).

Each plate was filled with 20 mL of minimal agar medium containing glucose and amended with a nitrogen source treatment and was inoculated with 1.35×10^7 spores of *A. sydowii*. One additional plate of each treatment served as a contamination control and was not inoculated. After inoculation, the plates were incubated at 25°C for 10 d. At the end of the incubation period, all fungal tissue was removed with a sterile wooden dowel, placed into 2-mL glass vials, and dried overnight at 60°C.

Table 1. $\delta^{15}\text{N}$ of *A. sydowii* isolates cultured on various nitrogen sources (nitrate, HSF, sea fan GS, and sea fan tissue). Isotope values are mean \pm standard error.

Isolate	Source	$\delta^{15}\text{N}$ (‰)				
		Experiment 1			Experiment 2	
		Nitrate alone	HSF+nitrate	HSF alone	GS+nitrate	Tissue+nitrate
AS16	Diseased <i>G. ventalina</i>	-1.1 \pm 0.1	3.6 \pm 0.1	6.8 \pm 0.3	0.8 \pm 0.3	5.1 \pm 0.4
AS17	Diseased <i>G. ventalina</i>	-3.0 \pm 0.1	3.3 \pm 0.2	5.3 \pm 0.1	-0.9 \pm 0.1	5.6 \pm 0.1
AS18	Diseased <i>G. ventalina</i>	-2.6 \pm 0.1	3.7 \pm 0.2	5.9 \pm 0.3	-1.3 \pm 0.2	4.7 \pm 0.3
AS35	Human clinical isolate	-1.9 \pm 0.1	3.9 \pm 0.1	6.8 \pm 0.2	0.1 \pm 0.5	5.5 \pm 0.3
AS72	Mangroves	-2.4 \pm 0.5	3.6 \pm 0.1	4.9 \pm NA	-1.8 \pm 0.1	4.6 \pm 0.2
Mean		-2.2 \pm 0.2	3.6 \pm 0.1	6.2 \pm 0.2	-0.5 \pm 0.3	5.1 \pm 0.1

Approximately 2.0 ± 0.15 mg of fungal tissue was weighed in 5×9 mm cups with a Sartorius MC5 microbalance. These samples were analyzed by the Cornell University Stable Isotope Laboratory (COIL) using a Finnigan MAT Delta Plus continuous flow isotope ratio mass spectrometer coupled to a Carlo Erba NC2500 elemental analyzer (EA-IRMS). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are relative to air and Vienna Pee Dee Belemite standards, respectively (IAEA). The precision of $\delta^{15}\text{N}$ measurements as determined by in-house standards (cabbage and methionine) was better than 0.08‰. Isotopic signatures of each nutrient source were previously measured ($\delta^{15}\text{N}$ homogenized sea fan = 4.0‰, $\text{NO}_3^- = 1.0$ ‰).

Values of $\delta^{15}\text{N}$ were tested for normality and homogeneity of variance before statistical analyses. A one-way ANOVA was used to test for the effect of nitrogen source on $\delta^{15}\text{N}$. When significant overall effects were found, Tukey's post hoc comparisons of means were performed to test for significant differences between pairs.

Stable isotope values of the fungus grown on the three nitrogen source treatments (nitrate alone, HSF alone, and HSF + nitrate; Table 1) were entered into a two-end member mixing model to calculate the proportion of sea fan biomass assimilated by *A. sydowii*:

$$\% \text{ sea fan N} = 1 - \left(\frac{\delta^{15}\text{N}_{\text{NO}_3^- + \text{HSF}} - \delta^{15}\text{N}_{\text{HSF}}}{\delta^{15}\text{N}_{\text{NO}_3^-} - \delta^{15}\text{N}_{\text{HSF}}} \right) \times 100$$

Because of differential fractionation of the HSF and nitrate end members, the isotope values of each isolate grown on the single sources were used in the mixing model (J. Sparks pers. comm.).

Experiment 2. Within-sea fan nitrogen preference—Gorgonin skeleton vs. homogenized sea fan tissue: To determine whether *A. sydowii* prefers sea fan gorgonin skeleton (GS) or tissue as a nitrogen source, the fungus was grown on agar media containing 40.0% glucose amended with GS + nitrate or tissue + nitrate. GS and tissue were separated by mortar and pestle from dried samples of healthy *G. ventalina* collected off the coast of Florida and were homogenized in a SPEX Certiprep cryogrinder with the use of liquid nitrogen before being added to the media. In

each treatment, the nitrogen contributed from each source was mass balanced. The resulting stable isotope values of these colonies were compared with values from colonies grown on GS alone, tissue alone, and nitrate alone. The experiment was conducted as previously described, including the use of the same five isolates of *A. sydowii*.

Stable isotope values of each isolate were entered into a two-end member mixing model, as previously described, to calculate the proportion of GS- and tissue-derived nitrogen assimilated by *A. sydowii*. A *t*-test was used to determine the effect of treatment on the proportion of sea fan-derived nitrogen assimilated by the fungus.

Pathogen variation in nitrogen assimilation: For each experiment, the proportion of sea fan-derived nitrogen assimilated by each isolate was compared by a one-way ANOVA and Tukey's post hoc comparisons. Additionally, the proportion of sea fan-derived nitrogen assimilated by sea fan isolates compared with non-sea fan isolates was compared by a one-way ANOVA and *t*-test.

Results

Experiment 1. Sea fan nitrogen assimilation: Organic vs. inorganic nitrogen preference—A. sydowii was able to grow on media amended with HSF as the only nitrogen source. All control plates had no fungal growth. The effect of nitrogen source on the $\delta^{15}\text{N}$ of *A. sydowii* was significant (ANOVA, $n = 62$, $F_2 = 829$, $p < 0.0001$). When both HSF and nitrate were present in the media, the $\delta^{15}\text{N}$ of the fungal tissue (mean $\delta^{15}\text{N} \pm \text{SE}$: 3.6 ± 0.18) was significantly higher than the $\delta^{15}\text{N}$ of fungi grown on nitrate alone (-2.2 ± 0.2) and significantly lower than values from fungi grown on HSF alone (6.2 ± 0.18 ; Fig. 1; Tukey's post hoc, $p < 0.05$), indicating that the fungi metabolized a combination of nitrate and sea fan-derived nitrogen.

A comparison between the nitrogen isotope values of nitrate and HSF sources and the resulting isotope values of fungi grown from those sources indicates differential isotope fractionation (Table 1). Fungi grown on HSF were on average 2.2‰ enriched relative to the HSF $\delta^{15}\text{N}$ (mean = 4.0‰). Conversely, fungi grown on nitrate were -3.2‰ depleted relative to the nitrate $\delta^{15}\text{N}$ (mean = 1.0‰).

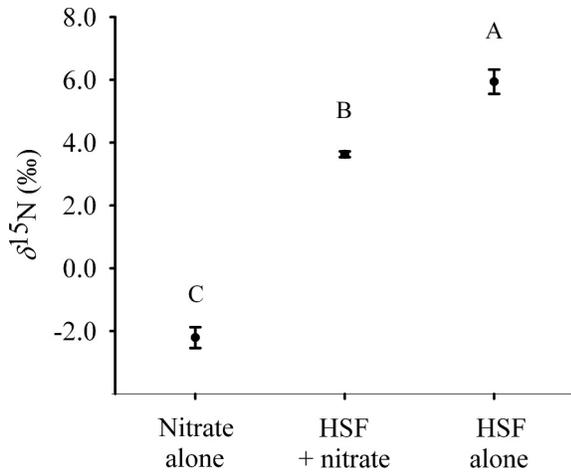


Fig. 1. Mean $\delta^{15}\text{N} \pm$ standard error of *A. sydowii* grown on three nitrogen source treatments: nitrate alone, HSF + nitrate, and HSF alone. Letters indicate significant differences between treatments (Tukey–Kramer post hoc comparisons, $p < 0.05$).

According to the mixing model, an average of $72 \pm 4.0\%$ (mean \pm SE) of the nitrogen assimilated by *A. sydowii* grown on media amended with HSF and nitrate was sea fan derived (Fig. 2).

Experiment 2. Within-sea fan nitrogen preference—Gorgonin skeleton vs. homogenized sea fan tissue: The proportion of sea fan-derived nitrogen assimilated was significantly different between the GS + nitrate and tissue + nitrate treatments (mean $\delta^{15}\text{N} \pm$ SE: -0.5 ± 0.29 , 5.1 ± 0.14 , respectively), with *A. sydowii* preferentially assimilating

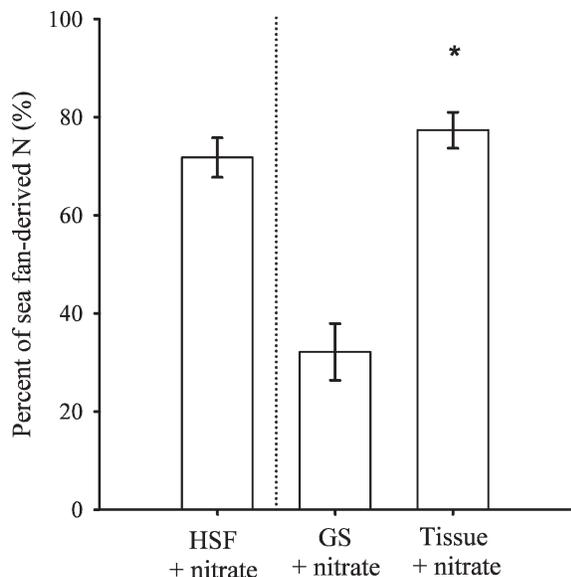


Fig. 2. Proportion of sea fan-derived nitrogen (vs. nitrate) assimilated by *A. sydowii* \pm standard error in three mixed treatments containing two nitrogen sources: a sea fan source (HSF, GS, tissue) and nitrate. The asterisk indicates a significant difference between the GS + nitrate and tissue + nitrate treatments (Tukey–Kramer post hoc comparisons, $p < 0.05$).

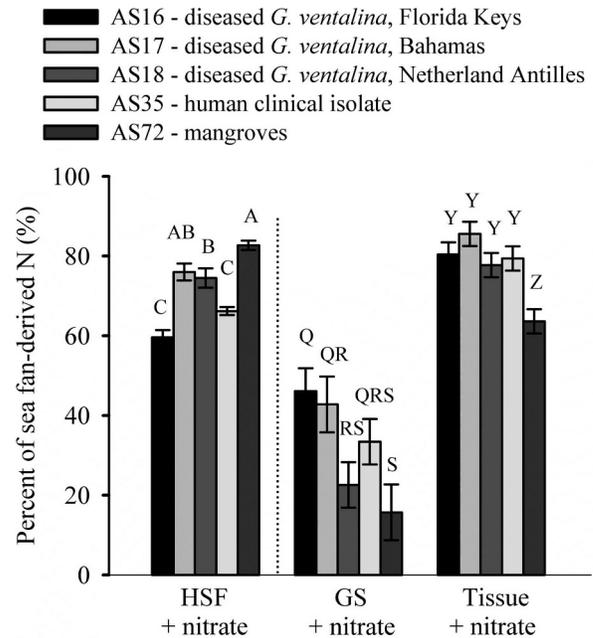


Fig. 3. Proportion of sea fan-derived nitrogen (vs. nitrate) assimilated \pm standard error for five *A. sydowii* isolates in three mixed treatments containing two nitrogen sources: a sea fan source (HSF, GS, tissue) and nitrate. Letters indicate significant differences between strains within treatments (Tukey–Kramer post hoc comparisons, $p < 0.05$).

nitrogen from sea fan tissue (Fig. 2; t -test, $n = 28$, $df = 26$, $t = 10.2$, $p < 0.0001$).

Pathogen variation in nitrogen assimilation: Comparisons between fungal isolates revealed significant differences with respect to the proportion of sea fan nitrogen assimilated by *A. sydowii* (59–82%) when grown on mixed media containing HSF and nitrate (Fig. 3; ANOVA, $n = 25$, $F_4 = 25.05$, $p < 0.0001$). Tukey’s post hoc comparisons revealed that the proportion of HSF nitrogen assimilated by AS72 (environmental isolate from mangroves) was highest, and AS16 and AS35 assimilated the least sea fan-derived nitrogen. The proportion of sea fan GS (vs. nitrate) assimilated ranged from 15% to 46% (Fig. 3). Significant differences were detected between isolates (ANOVA, $n = 13$, $F_4 = 4.16$, $p < 0.04$), with one disease-causing isolate (AS16) assimilating significantly more nitrogen from sea fan GS than strains AS18 and AS72 (post hoc pairwise t -test, all $p < 0.05$). Proportion of sea fan tissue (vs. nitrate) assimilated ranged from 61% to 89%. Significant differences were also detected between isolates (ANOVA, $n = 15$, $F_4 = 7.23$, $p < 0.005$), with AS72 assimilating significantly less nitrogen from sea fan tissue than any of the other four strains (post hoc pairwise t -test, $p < 0.05$).

When *A. sydowii* isolates were grouped according to source (diseased sea fan or not), the proportion of sea fan nitrogen assimilated in the HSF + nitrate and GS + nitrate treatments showed no effect of source. The proportion of sea fan nitrogen assimilated in the tissue + nitrate treatment showed an effect of source (t -test, $n = 15$, $df = 13$, $t =$

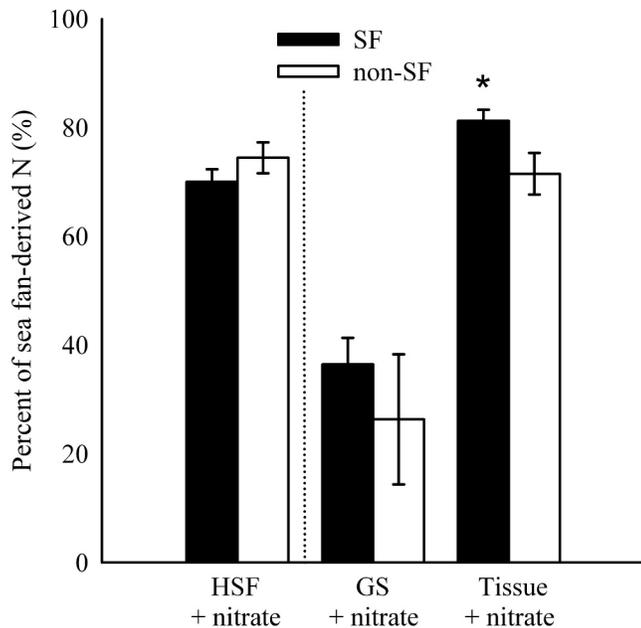


Fig. 4. Proportion of sea fan-derived nitrogen (vs. nitrate) assimilated \pm standard error for isolates of *A. sydowii* cultured from sea fan (SF) and non-sea fan (non-SF) substrates in three mixed treatments containing two nitrogen sources: a sea fan source (HSF, GS, tissue) and nitrate. The asterisk indicates a significant difference between the GS and tissue treatments (Tukey–Kramer post hoc comparisons, $p < 0.05$).

-2.43 , $p = 0.03$), confirming that sea fan-derived isolates assimilated significantly more nitrogen from sea fan tissue than other isolates (Fig. 4).

Discussion

Despite extensive knowledge of the physical signs (Smith et al. 1996), host immune response (Mydlarz et al. 2008), and environmental correlates of aspergillosis (Baker et al. 2007), the metabolism of sea fan-derived nutrients had not been tested before this study. In this experiment, $\delta^{15}\text{N}$ of fungi grown on media containing HSF were on average 1.9‰ higher than the HSF $\delta^{15}\text{N}$ (4.0‰; Fig. 1), indicative of trophic enrichment, likely because of retention of ^{15}N during protein catabolism and deamination (Minagawa and Wada 1984; Macko et al. 1986). Thus, we demonstrate that *A. sydowii* is capable of catabolizing *G. ventalina*. Furthermore, the $\delta^{15}\text{N}$ of nitrate and $\delta^{15}\text{N}$ of *A. sydowii* grown on nitrate showed a pronounced difference. The -3.2 ‰ depletion between nitrate and the fungus suggests strong discrimination against ^{15}N during the active transport of inorganic nitrogen. Fractionation of nitrogen isotopes during nitrate uptake has been observed in plants when the concentration of nitrate is high relative to the concentration of nitrate reductase, which can occur in young plants with low enzyme concentrations or under high nitrate concentrations (Mariotti et al. 1982). Therefore, the isotopic depletion we observed between nitrate and fungus suggests that the fungus was enzyme limited. Although these fractionations do not affect our conclu-

sions, they allude to the mechanisms by which *A. sydowii* obtains nitrogen from the environment.

More than half of nitrogen assimilated by *A. sydowii* in the HSF + nitrate treatment was sea fan derived, so the hypothesis that *A. sydowii* prefers nitrate was not supported. *A. sydowii* prevalence has been found to correlate with increased TN concentrations (Baker et al. 2007). However, given our results, it is unlikely that inorganic nutrients are the sole driver of this relationship. In the Florida Keys, $> 85\%$ of the TN pool is dissolved organic nitrogen (DON; Boyer and Briceno 2008). Thus, organic nitrogen might be more important for driving disease outbreaks, in that *A. sydowii* is able to assimilate organic nitrogen and does so preferentially over nitrate. *A. sydowii*'s ability to assimilate sea fan-derived carbon might also affect disease dynamics. An additional mass-balanced experiment could be conducted to determine fungal preference for glucose or sea fan-derived carbon.

From an energetic perspective, sea fan nitrogen might be a more costly energy source because accessing and assimilating the organic nitrogen in the sea fan requires the breaking of more bonds than transporting nitrate across the cell membrane. However, the cost of assimilating sea fan nitrogen might be offset by the benefit of acquiring essential amino acids. Solomon et al. (2003) suggest that phytopathogenic fungi need host-derived amino acids to propagate within host plants. If certain amino acids are not available in high enough concentrations, the fungus must synthesize them. Given that elevated levels of nitrogen in the water column increase fungal growth (E. Rivest unpubl.) and the prevalence and severity of aspergillosis (Bruno et al. 2003; Baker et al. 2007), *A. sydowii* might be able to take advantage of multiple sources of nitrogen in its environment. By using the inorganic nitrogen available in the water column, this pathogen might be able to supplement its nutrition, allowing it to allocate its energy for acquiring only essential forms of nitrogen from its host (such as amino acids).

Our study suggested that *A. sydowii* derives more nitrogen from its sea fan host than from nitrate in the water column. Within the sea fan, *A. sydowii* prefers to assimilate nitrogen from sea fan tissue over GS. Thus, our hypothesis that *A. sydowii* prefers sea fan tissue as a nitrogen source is confirmed. These results complicate our understanding of the *A. sydowii*–*G. ventalina* pathosystem, given previous evidence that suggests most infections establish in the gorgonin skeleton of the sea fan (Ellner et al. 2007). A critical next step is to explore the mechanism of nutrient acquisition used by this fungal pathogen. It is known that sea fans produce antifungal compounds in the tissue (Kim et al. 2000), which might explain why *A. sydowii* is found in the non-cellular gorgonin skeleton during infection. However, to metabolize and acquire nitrogen from sea fan tissue, the fungus would need to evade or overcome sea fan immune defenses.

Isotopic evidence as well as fungal growth on sea fan-amended media support the hypothesis that, as a species, *A. sydowii* can catabolize sea fan nitrogen and convert it to fungal biomass as predicted by Ellner et al. (2007). It is important to note that the ability of fungal isolates to

assimilate nutrients from HSF does not confer the ability to cause disease. A successful infection is the result of both host immunity and pathogen virulence. Sea fans produce antifungal compounds as part of an inducible immune response to *A. sydowii* (Kim et al. 2000); these compounds are likely inactive in the treatments used here. Previous studies have observed that natural levels of sea fan defenses should be sufficient to prevent infection by *A. sydowii* (Dube et al. 2002). However, stressors such as increased ocean temperatures or physical damage could render the sea fan more susceptible to infection (Harvell et al. 1999). While the observation that the five *A. sydowii* isolates tested are capable of assimilating sea fan nutrients is not indicative of their virulence, it does confirm their ability to grow successfully using only the nutrients provided by HSF.

The five *A. sydowii* isolates tested showed variable responses in the proportion of sea fan nitrogen assimilated (Fig. 3). *A. sydowii* seems to be a true opportunist with all isolates able, though unequally, to use nutrients available in sea fans. Variation among isolates in the proportion of sea fan-derived nitrogen assimilated could be the result of adaptive population differentiation. A previous study of *A. sydowii* that used neutral microsatellite markers found that fungi isolated from both coral and environmental substrates from around the world form a single genetic population (Rypien et al. 2008). However, given the diversity of substrates *A. sydowii* can colonize, isolates might exhibit ecological specialization, selected for by the conditions of their environment (Verhoeven et al. 2004). For example, if AS72 from mangroves was originally isolated from a nutrient-rich area, its decreased assimilation of sea fan-derived nitrogen might be a result of local adaptation to its previous, non-limiting environment. To determine whether adaptive population differentiation is a likely explanation for the observed variation between isolates, the prevalence and severity of aspergillosis as well as nutrient concentrations in the water column at the time of fungal isolation should be examined. Comparing *A. sydowii* isolates from diseased sea fans to those from other sources provides some indication that fungi isolated from diseased *G. ventalina* have distinct nitrogen metabolism because they assimilated significantly more sea fan tissue nitrogen than isolates from other sources (Fig. 4). Additionally, sea fan isolates, when pooled, demonstrated a trend toward higher levels of nitrogen assimilation from sea fan GS than the non-sea fan isolates, although this difference was not statistically significant. This suggests that the ability to use tissue- and GS-derived nitrogen might be an advantage for infection. Future studies should examine the mechanism of nutrient metabolism in *A. sydowii*—for example, differential enzymatic activities of nitrate and nitrite reductases and extracellular proteases when grown on multiple nitrogen sources (Marzluf 1997).

Understanding the link between anthropogenic factors and coral disease dynamics is crucial for the successful management of disease-causing factors. Elevated nitrogen concentrations in the environment are occurring more frequently at a global scale (Lotze et al. 2006) and are a

major influence on coral reef decline. Stable isotope experiments demonstrate that *A. sydowii* can use sea fan-derived matter to produce biomass. The fungus prefers organic nitrogen from sea fans over nitrate, with more nitrogen assimilated from sea fan tissue than from sea fan GS. *A. sydowii* isolated from diseased sea fans assimilate more tissue-derived nitrogen than fungi isolated from other sources.

This study improves our understanding of the complex role of nutrient conditions on the *A. sydowii*–*G. ventalina* pathosystem, the role of nutrient enrichment as a disease driver, and models of infection (Ellner et al. 2007). Foci of future research should include (1) the interaction between growth of *A. sydowii* and preference for nitrogen sources, (2) the role of DON in aspergillosis prevalence and severity, (3) potential DON thresholds as management targets, (4) molecular characterization of organic nitrogen assimilation mechanisms, and (5) growth rates of *A. sydowii* on various nitrogen sources and concentrations of DON.

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