Tardigrade response to nitrogen and phosphorus addition

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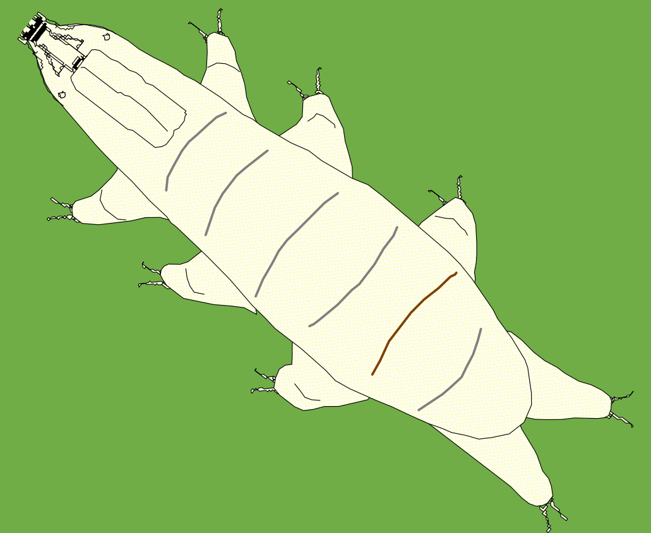
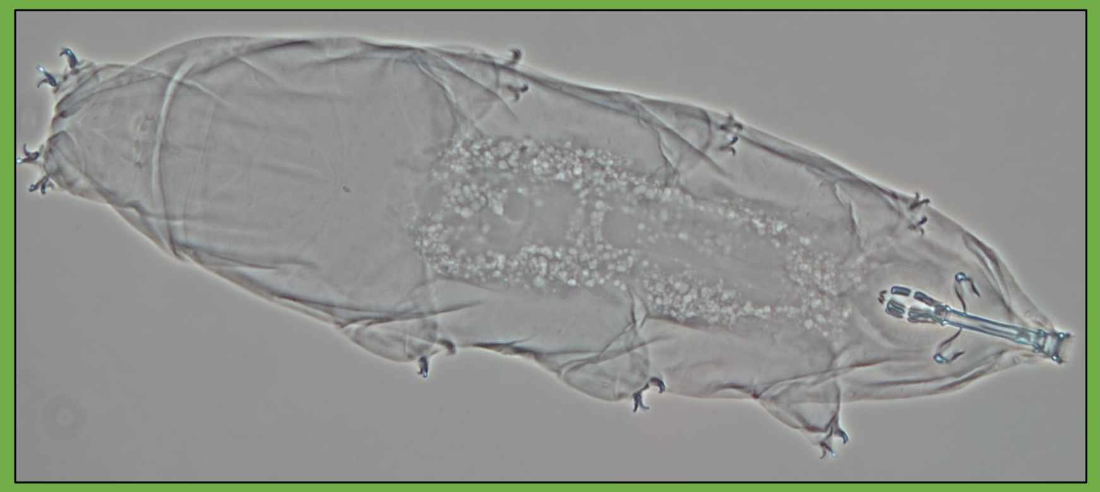




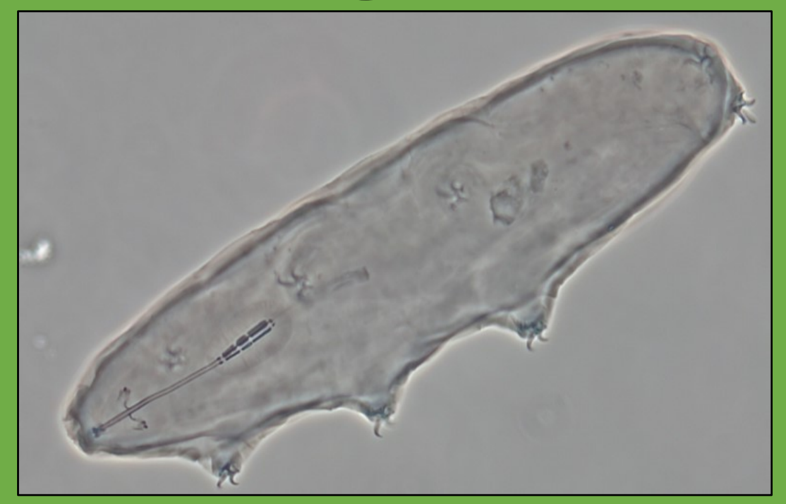
Macrobiotus sp.2.1
Found in C5, plot 1

Doryphoribius sp.
Found in C6, plot 4

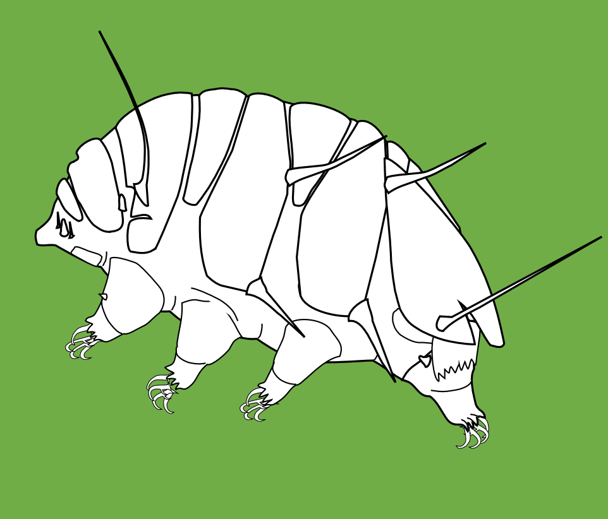
Hypechiniscus gladiator
Found in C7, plot 3

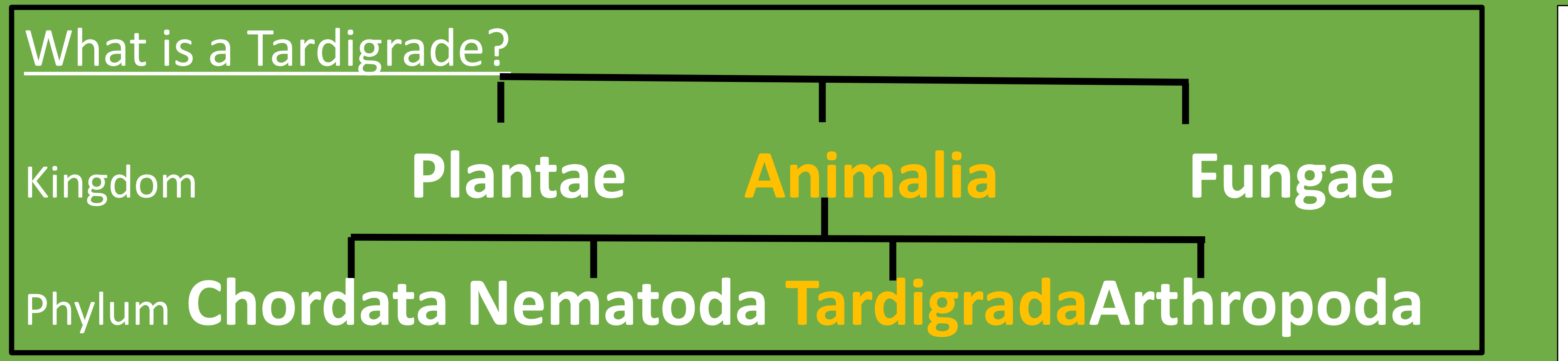
Diphascon pingue
Found in C9, plot 1

Calohypsibius ornatus
Found in C3, plot 4



Adropion scoticum
Found in C1, plot 2



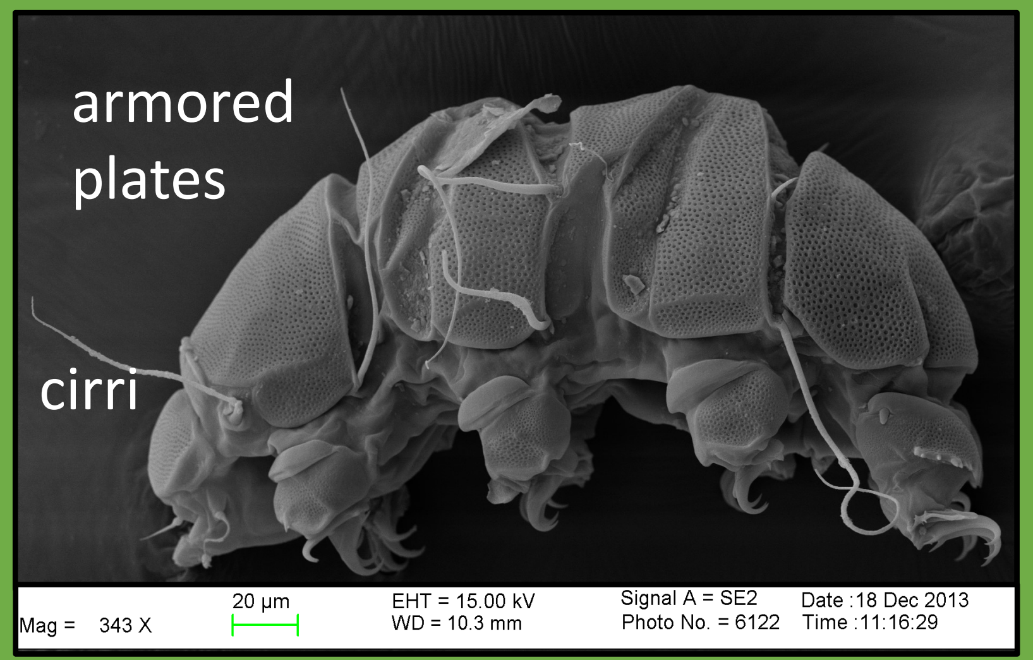


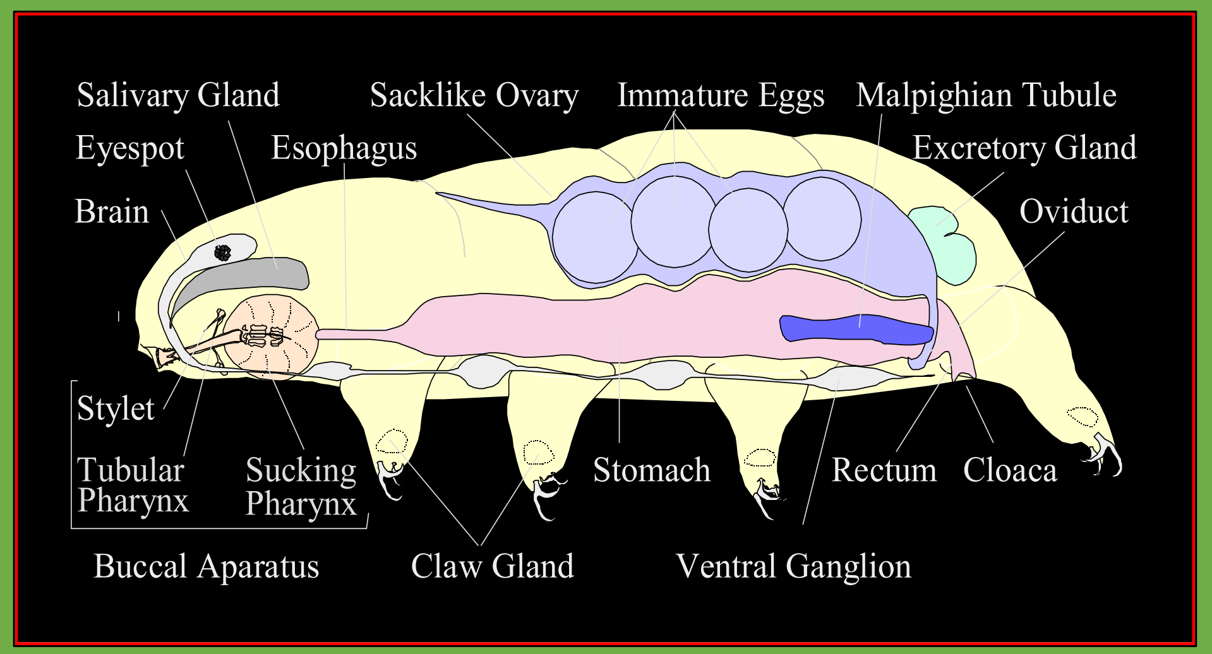
What is a Tardigrade?

Kingdom **Plantae** **Animalia** **Fungae**

Phylum **Chordata** **Nematoda** **Tardigrada** **Arthropoda**

Introduction: Tardigrades are small (0.2-1 mm) animals that can be found on every continent, in a wide variety of ecosystem types. Tardigrades prey on microbial communities that are likely limited by nutrient availability. However, no studies to date have examined the effect of nutrient additions on tardigrade communities. Here, we assess the response of tardigrade communities to fertilization with nitrogen and phosphorus across nine northern hardwood forest stands.





Methods: In August 2017, we collected 108 samples of moss from rock surfaces in the Bartlett Experimental Forest in the White Mountains of New Hampshire, USA, where research plots had been fertilized annually since 2011 with nitrogen, phosphorus, a combined N+P, and a control with no fertilizer. Three patches of moss were collected per plot and dried at room temperature. One gram of oven dry moss was hydrated with 20 ml of water, then searched for tardigrades under a dissecting scope. An Irwin loop was used to transport individual specimens onto a glass slide with PVA mounting medium then covered with a glass coverslip. Tardigrades were identified to species level using phase-contrast microscopy and taxonomic keys.

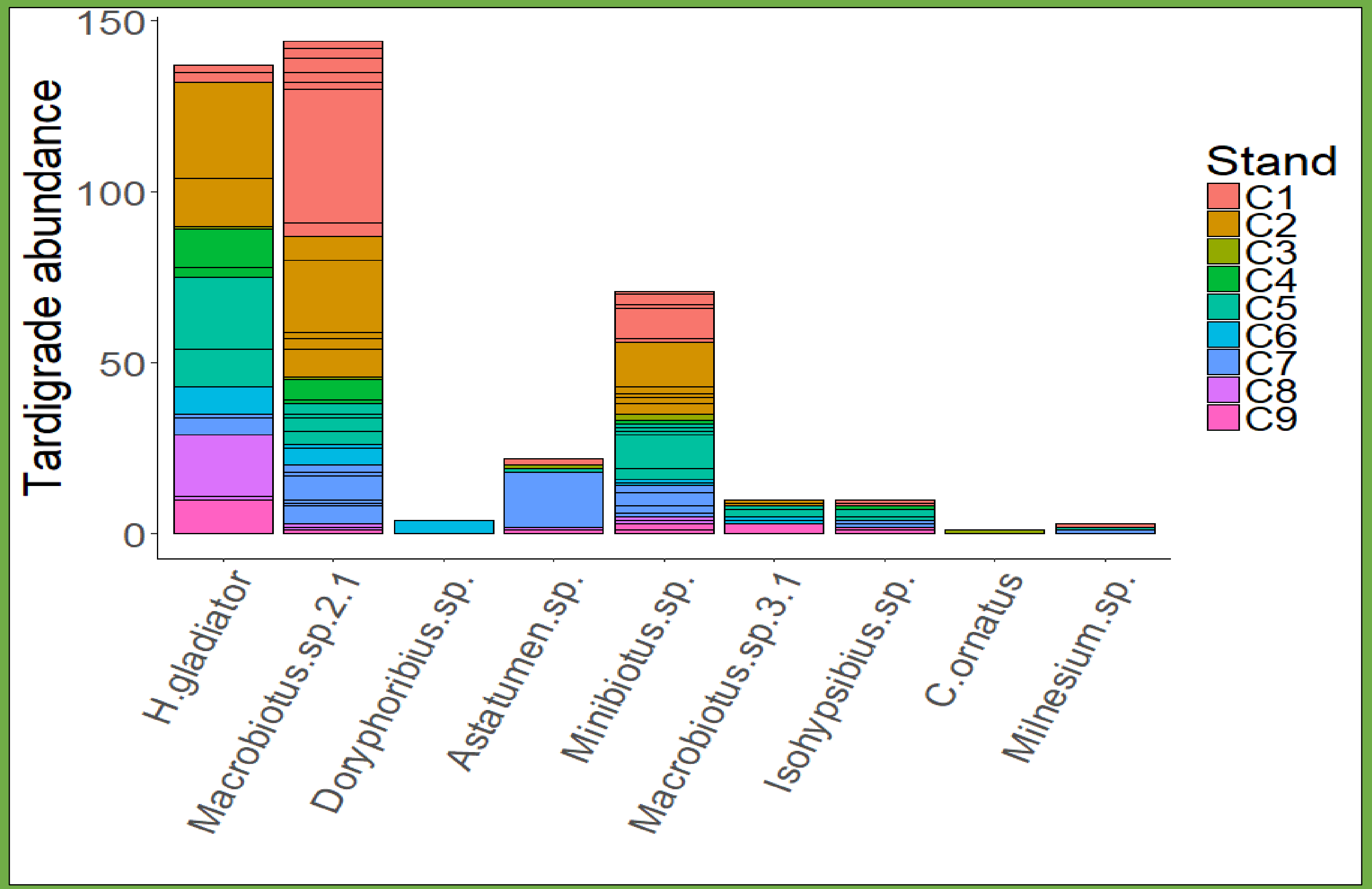


Figure 1. Of the nine morpho-species identified, *H. gladiator and Macrobiotus 2.1* were most common. In contrast *C. ornatus* was only found once in one sample.

Figure 2. (Left) an example plot layouf of stands C1 and C5, each plot is 50 by 50 square meters. (Right) A tardigrade laying eggs in its recently shed cuticle, six eggs can be seen.

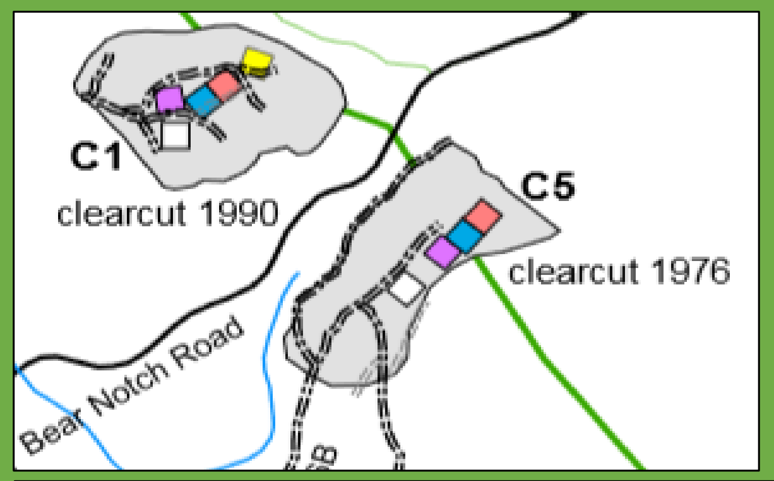


Table 1. Average tardigrade abundance in each treatment.

Class, Order, Superfamily, Family	Genus species	Control	Nitrogen	Phosphorus	N+P
Heterotardigrada, Echiniscoidea, Echiniscoididae	Hypechiniscus gladiator	**11.3**	**9.2**	**3.3**	**15**
Eutardigrada, Apochela,Milnesiidae	Milnesium sp.			**1**	
Eutardigrada, Parachela, Hypsibioidea, Hypsibiidae	Calohypsibius.ornatus			**1**	
	Doryphoribius sp.				**4**
Eutardigrada, Parachela, Hypsibioidea, Itaquasconinae	Astatumen sp.		**2**	**4**	
Eutardigrada, Parachela, Isohypsibidea, Isohypsibioiidae	Isohypsibius sp.		**2**	**1**	
Eutardigrada, Parachela, Macrobiotoidea, Macrobiotidae	Macrobiotus sp. 2.1	**3.6**	**9.2**	**2**	**7**
	Macrobiotus sp.3.1	**3**	**2**	**1**	
	Minibiotus sp.	**3**	**2**	**1.6**	**9**

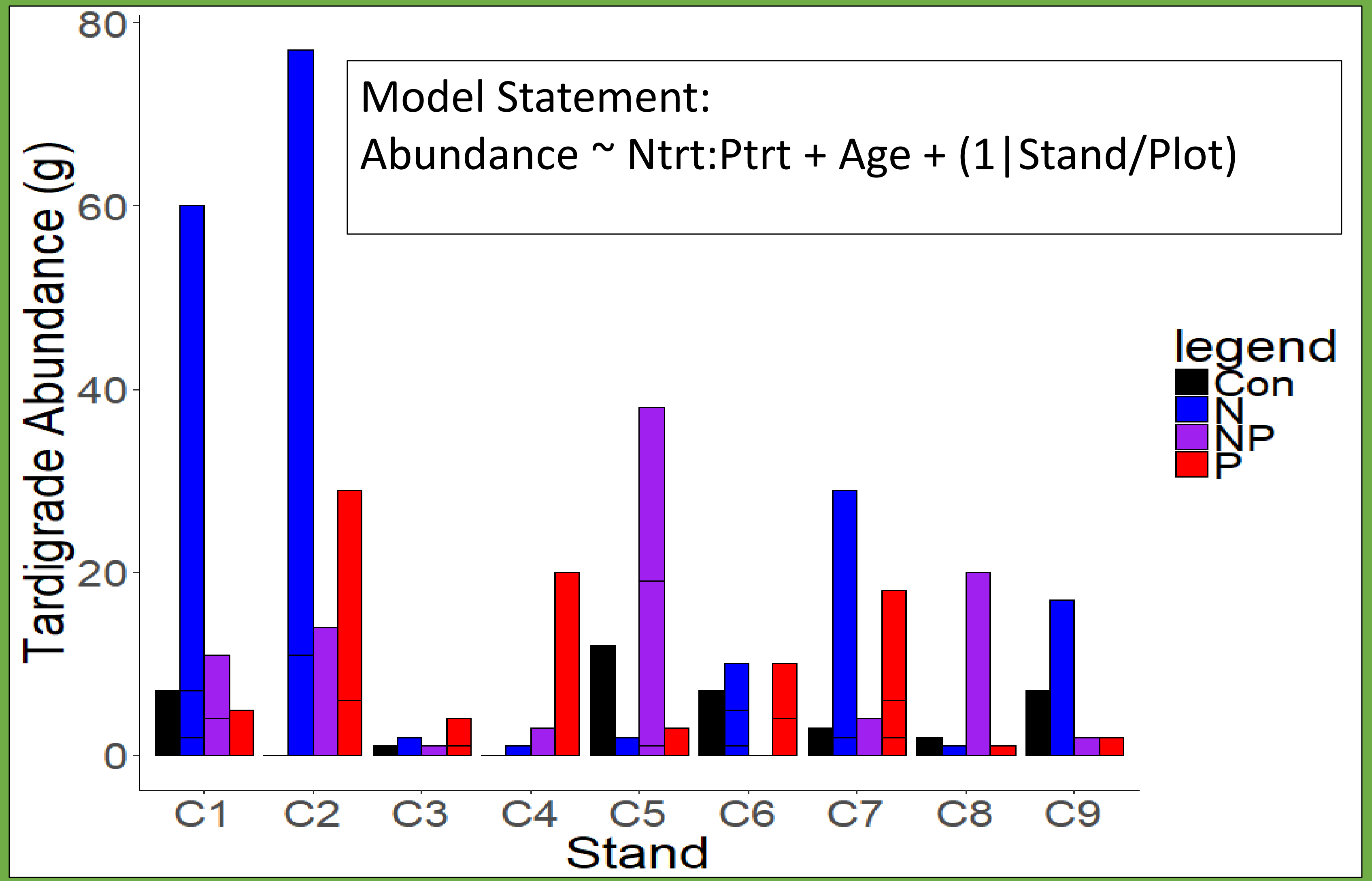


Figure 3. Tardigrade abundance increased in both N and NP treated plots having 28% more tardigrades compared to plots not amended with N (*p* = 0.05).

Discussion Tardigrade communities may respond to nutrient additions due to changes in their food source or changes in other factors affecting their population dynamics, such as migration, fecundity, longevity, or mortality. Interestingly, tardigrade community composition did not respond to fertilization (p = 0.18). The impact on tardigrade abundance may have cascading effects throughout forest ecosystem food webs.

Future studies could investigate the role of tardigrades as bioindicators of microclimate conditions, particularly with regard to nutrient availability. For example, a comparison of areas with high and low nitrogen deposition and the resulting impact on tardigrade communities would further demonstrate tardigrade community response to the elevated levels of nitrogen.

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