Mycorrhizal fungi colonization and community dynamics in a long-term rotation

as influenced by preceding crop and weed management intensity

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Objectives

To examine root growth, AM fungal colonization and AM fungal community structure in a durum wheat trap crop over time grown in soils with different preceding crops (flax or canola) and weed seedbank densities (Low, Medium, High) collected from a long-term experiment.

Background

In 1999, a study investigating the effects of reduced in-crop herbicide use (Pesticide Free Production) was initiated at the University of Manitoba. This experiment contains an annual crop rotation (flax-oats-canola-wheat) under no-till production that is replicated three times in each block. Each repetition of the rotation is treated with a different level of in-crop herbicide use intensity including: i) no in-crop herbicide omissions, ii) in-crop herbicide omissions in oats only, & iii) in-crop herbicide omissions in oats & flax. These have resulted in weed seedbank densities of about 5000, 7000, and 11000 seeds m^{-2}, respectively (Gulden et al. 2013).

Plants directly influence soil microbes which in turn affect soil processes. Arbuscular mycorrhizal (AM) fungi are obligate symbionts that assist with plant nutrient uptake, stress tolerance and facilitate soil processes (Willis et al. 2013). The presence of weeds can influence AM fungi and in non-mycorrhizal crops such as canola, weeds are the only plants that can host AM fungi. The dominant weed species in the long-term PFP study include green (*Setaria viridis* L.) and yellow foxtail (*S. glauca* L.), red root pigweed (*Amaranthus retroflexus* L.), yellow wood sorrel (*Oxalis stricta* L.) and a number of minor weed species belonging to the Asteraceae, Chenopodacea, Polygonacea and Brassicaceae families (Gulden et al. 2010). The dominant weeds in this study are not highly mycotrophic.

Weed seedbanks are a memory of weed populations and management practices in an agricultural system (Cavers 1995). More recently it has been suggested that weed seedbanks contribute to soil function (Franke et al. 2009), however, aside from hosting nematodes and some pathogens, little is known about the associations between weed seeds and soil function.

Methods

After harvest in 2011, soil was collected from the flax and canola treatments to a depth of 15cm, homogenized and used in a growthroom study where durum wheat was grown as a trap crop in these soils. No weed were allowed to grow during this experiment. Pots were watered regularly and carefully to minimize collection of water and leached nutrients into saucers.

Durum wheat was harvested at four stages of development (3-leaf, 5-leaf, Flowering & Physiological Maturity) and the following response variables were determined at each harvest:

Durum root and shoot dry matter and total root length. Root length was determined using digital image analysis (Assess 2.0, APS, St. Paul, MN)
AM fungal colonization – grid-intersect method (McGoniggle et al. 1999)
AM fungal community structure – T-RFLP analysis on AM fungal specific amplicon (Lee et al. 2008). Cloning and sequencing of T-RF fragments is still to be conducted.

Univariate data were analysed using Mixed models in SAS, while AM fungal community structure was subjected to PCA analysis before univariate analysis of significant individual principal components. Assumptions of ANOVA were tested (outliers, residuals, heterogeneity of variance) prior to final analysis and means were separated using Fisher's protected LSD. Covariance analysis with initial soil characteristics (pH, OM, Olsen P, NH4, NO3, total N) was conducted.

Fixed effects:	Preceding crop (flax or canola)
	Weed seedbank density (Low , Medium, High)
	Durum developmental stage (3-leaf, 5-leaf, Flowering, Physiological Maturity)
Random effect:	Block

Season-long, total colonized root length was calculated by multiplying colonization by root length at each harvest date and integrating this over time using the area under the disease progress curve approach (Wilcoxson et al. 1974). To determine whether AM fungal types that colonized first also were more abundant at later developmental stages, single-degree-freedom contrasts were designed to compare the average relative abundance of the group of T-RFs that were present at the 3-leaf stage to the average relative abundance of the group of T-RFs that were detected only after the 3-leaf stage at flowering and physiological maturity than those that were detected only after the 3 leaf stage.

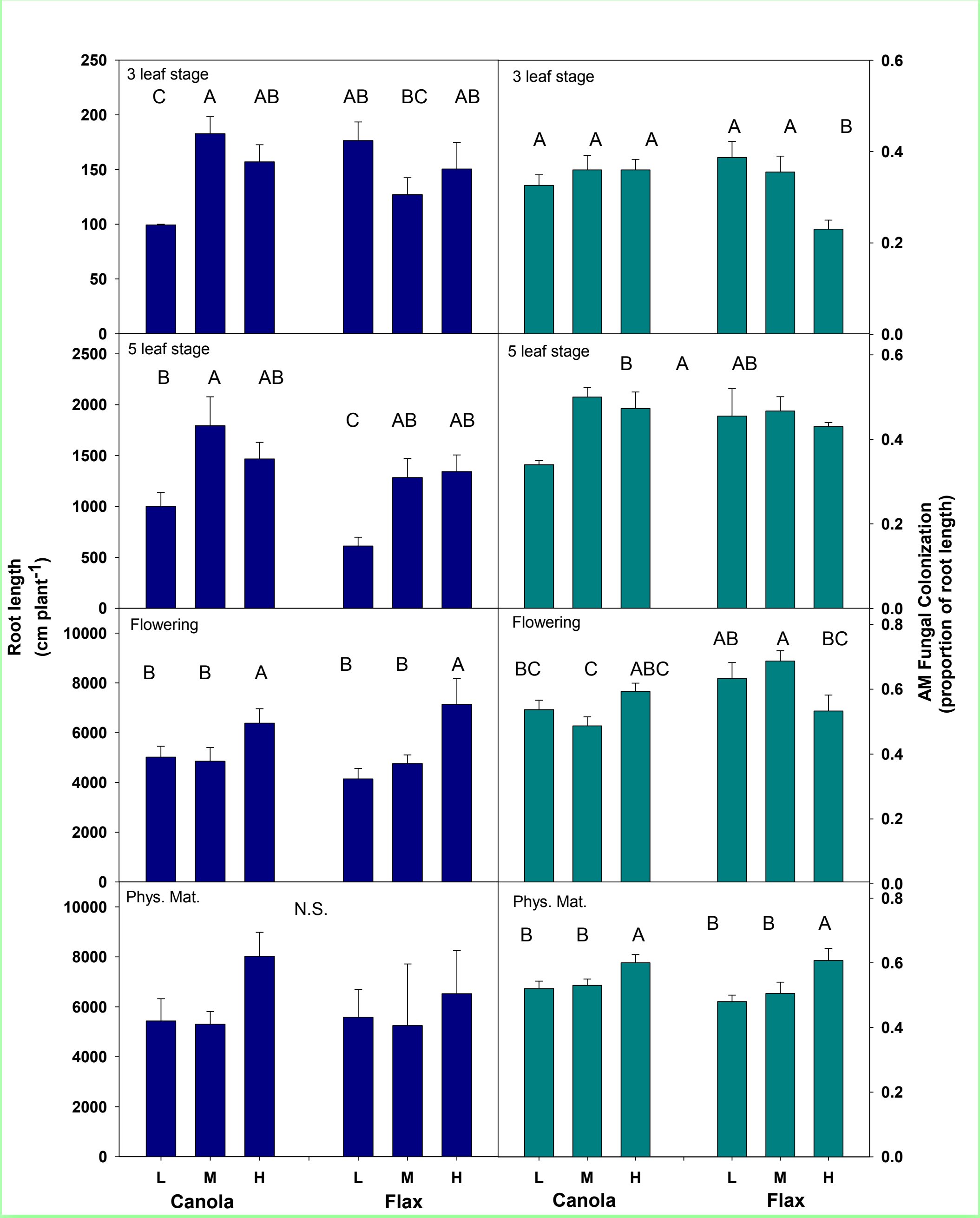

Figure 1 **Durum root length (left) and root colonization by AM fungi (right) in response to preceding crop and weed seedbank density at the 3-leaf (top), 5-leaf (middle top), flowering (middle bottom) and physiological maturity (bottom) developmental stages. Different letters above bars indicate significant differences between means as determined by Fisher's protected least significant difference (alpha = 0.05).**

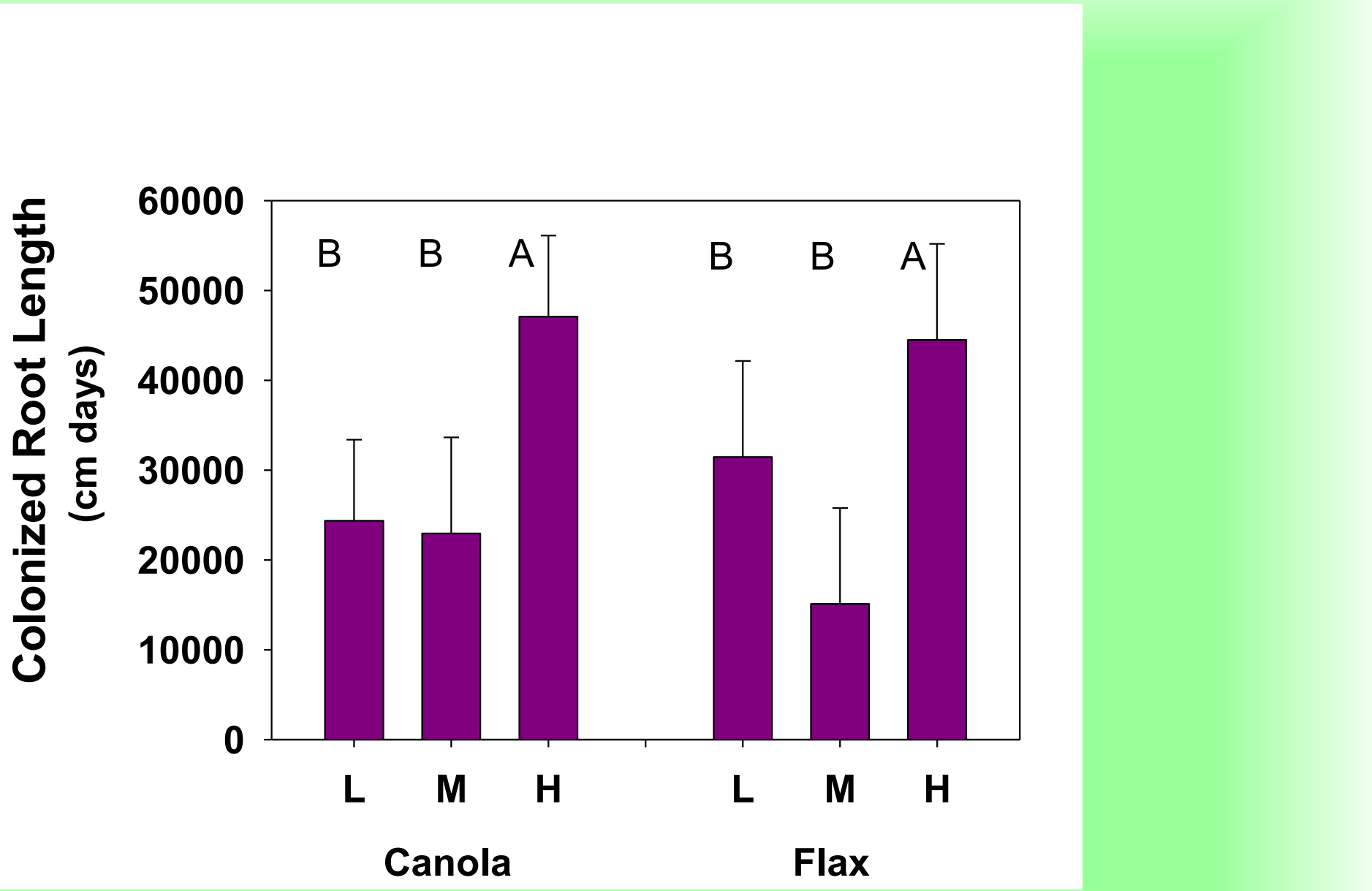

Figure 2 **Cumulative root length colonized by AMF throughout the development of durum wheat as influenced by preceding crop and weed seedbank density (L, M, H). Different letters above bars indicate significant differences between means as determined by Fisher's protected least significant difference (alpha = 0.05). Three letters separating groups of bars indicate a weed seedbank density main effect only.**

Summary

AM fungal colonization and community structure in durum wheat grown in soils retrieved from this long-term study showed that subtle changes in weed management had significant effects on durum root growth and the dynamics of the AM fungal community. Increased weed seedbank densities contributed to greater root exploration of the soil through increased root length and root length colonized by AM fungi over the entire life cycle of durum wheat and these observations were not linked to initial soil nutrient status. The magnitude of the effects of weed seedbank density was similar to or greater than those linked to preceding crop (flax or canola) which, in this study, had inherently divergent mycotrophic potential. The observed effects related to weed seedbank densities may have been even more pronounced if the dominant weed species were more mycotrophic. Weed seedbank density and preceding crop both resulted in different AM fungal community trajectories throughout the development of durum wheat. These differences were driven by differences in community richness and the relative abundance of fungal types. On average, early colonizing fungal types tended to be more abundant throughout the life cycle of wheat, although this was not universal for all fungal types. Our findings clearly showed that field management history over the longer-term is an important factor that contributes to AM fungal presence, community structure and dynamics in conventional agriculture systems. To better understand AM fungal community structure and dynamics in these systems, there is a need to better document weed populations, weed seedbanks and field management history.

Results & Discussion

Relatively subtle differences in weed management, in specific, reduced in-crop weed management that over a period of ten years resulted in stable differences in weed seedbank densities among the treatments (Gulden et al. 2011), were associated with altered root exploration of the soil and different colonization and intra-radicle AM fungal community dynamics in a durum wheat test crop. In this study, effects of weeds were related to weed seedbank densities and not above ground weed population densities. Above ground weed population were substantially different in canola compared to flax. In flax, the above ground weed populations reflected seedbank densities, however in canola, effective in-crop herbicides in addition to the competitive ability of canola resulted in virtually complete control of weeds in all treatments, irrespective of initial weed seedbank density.

In this growthroom study, durum root and shoot dry matter were affected by both increasing weed seedbank density and preceding crop at the 3- and 5-leaf vegetative stages, however, these differences were no longer apparent at the reproductive stages (data not shown). Durum shoot dry matter was closely related to root dry matter (Pearson R = 0.71) and root dry matter was closely related to root length (Pearson R = 0.87) (Fig. 1). The proportion of durum wheat root length colonized by AM fungi was influenced principally by durum developmental stage, however, some differences in response to preceding crop and weed seedbank density were observed within developmental stage. High weed seedbank densities promoted increased cumulative root length and cumulative colonized root length by AM fungi (Fig. 2). Covariate analysis indicated that initial soil conditions or nutrient status did not influence related to durum root growth or AM fungal colonization response variables (data not shown). For durum wheat and AM fungal colonization response variables, most of the variance (based on sums squares) was partitioned to durum developmental stage (65-97%) and only a small portion of the total variance was consumed by preceding crop and weed seedbank density effects. Root dynamics and architecture respond to many factors including soil biota (de Kroon et al. 2012) and weed seedbank densities affect some soil microbiota (Franke et al. 2009). It is possible that altered soil biota contributed to the root dynamics observed at high seedbank densities. Positive relationships between root length, root colonization and AM fungal inoculum have been reported before (Padilla and Encina 2005; Wu et al. 2012). The opposite, however, has been documented as well (Isobe et al. 2002). Our results agreed with the former as a trade-off between root length and colonization by AM fungi was not observed.

The AM fungal community structure was determined at the each developmental stage of durum wheat. A total of 20 T-RFs were identified that were included in the statistical analysis. PC analysis of the AM fungal T-RFs revealed 6 influential principal components (PCs) (Eigenvalues > 1), however, based on univariate statistical analysis and amount of total variation attributable to each PC, only three of the six PCs effectively contributed to defining the differences in the AM fungal community in response to preceding crop, weed seedbank density, durum developmental stage and their interactions (Table 1). The structure of the intraradicle community AM fungal community was affected by durum developmental stage, weed seedbank density and preceding crop.

Beginning with similar AM fungal community structure at the 3-leaf stage, AM fungal communities followed different trajectories over the development of durum wheat in response to preceding crop and weed seedbank densities (Fig. 3 middle, bottom). Different AM fungal communities and trajectories were also observed in response to seedbank and preceding crop (Fig. 1 top). Community differences between preceding crop were largely driven by differences in community richness with greater richness after preceding flax than preceding canola. The different community trajectories in response to weed seedbank densities were driven by differential abundance of dominant and subdominant fungal types. Seasonal changes in intra-radicle AM fungal communities are common in agricultural (Daniell et al. 2001) and natural systems (e.g. Davison et al. 2011), however, the causes of these dynamics are only beginning to be understood. In this growthroom study, differences in AM fungal community structure over time were driven by factors related to host developmental stage as daylength and temperature did not change throughout the experiment. At flowering and at physiological maturity, AM fungal T-RFs that were first to colonize the roots of durum wheat (observed at the 3-leaf stage vs. later) also were on average more abundant at these later developmental stages (data not shown).

Preceding canola resulted in fewer AM fungal types, in particular, sub-dominant types. This was not unexpected given the mycorrhizal dependence of flax (Monreal et al. 2012) and the non-mycotrophic nature of canola. All T-RFs detected after canola were a nested subset of the T-RFs identified after preceding flax and did not include any T-RFs that were unique to preceding canola. The non-mycotrophic nature of canola selected for more persistent, resilient, or dominant AM fungal types. In the preceding canola crops, the absence of weed biomass, irrespective of weed seedbank densities resulted in limited opportunity for AM fungi to colonize the same weed hosts that were present in the preceding flax crop. The importance of weeds and their species complement to maintaining AM fungi in cropping systems is being recognized (e.g. Ramos-Zapata 2012). Nested AM fungal communities have been reported among organic and conventionally managed fields with a reduction in richness in more disturbed and less diverse systems (Verbruggen et al. 2012).

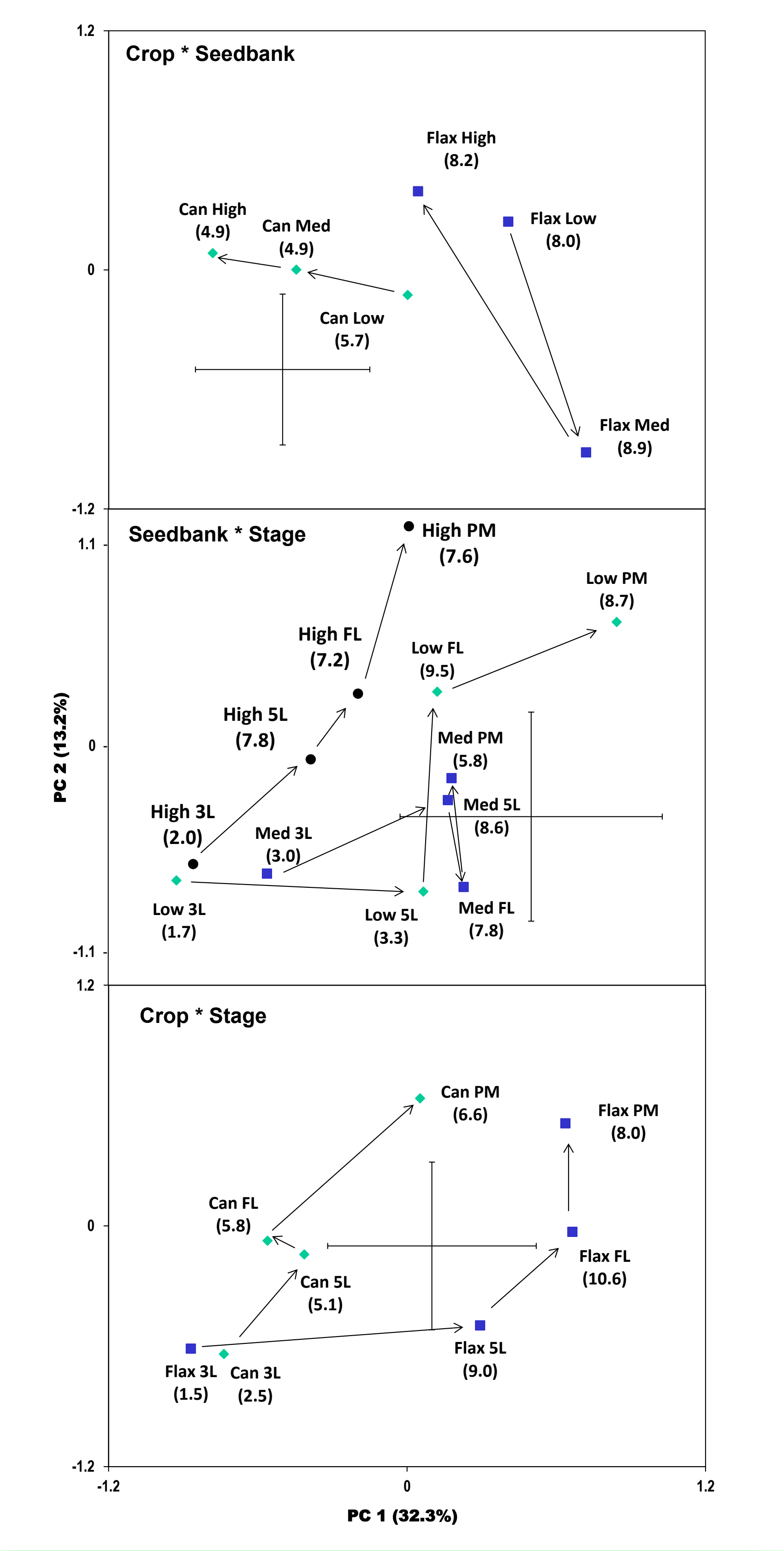

Figure 3 **Biplot of PC1 and PC2 of the rotated factor pattern of the individual T-RFs of the intraradicle AM fungal community in the durum wheat test crop. Treatment means of preceding crop by seedbank density (top), seedbank density by developmental stage (middle) and crop by developmental stage (bottom). Arrows indicate treatment trajectories over seedbank densities (top) and developmental stages (middle, bottom). T-RF richness (in parentheses) and Fisher's protected LSD for PC 1 and PC 2 are indicated on each panel.**

Table 1**. Percent total variation and p-values for preceding crop (Crop), weed seedbank density (Weeds), durum developmental stage (Stage) main effects and their interactions for PC 1 through PC 6 as determined by mixed model ANOVA and the average contribution of each effect to the total variance as determined by the percentage of sums squares allocated to each effect for PC 1 to PC 6. Significant p-values (< 0.05) are indicated in bold and standard errors of the means are indicated in parentheses.**

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	Variance components
% Total Variation	32.3	13.2	8.7	6.9	6.1	5.3	
	---------------------------------- p-value			----------------------------			% variation
Effect							
Crop	**0.003**	0.292	0.138	0.676	0.117	0.388	**3.2 (1.3)**
Weeds	0.319	**0.045**	0.290	0.216	0.748	0.706	**2.9 (0.9)**
Crop*Weeds	0.403	**0.005**	0.693	**0.001**	0.979	0.258	**4.9 (2.4)**
Stage	**0.008**	**0.001**	0.190	0.056	0.472	**0.040**	**11.5 (3.0)**
Crop*Stage	0.156	0.742	0.645	**0.049**	0.671	0.572	**3.3 (0.7)**
Weeds*Stage	0.440	0.460	0.251	**0.001**	0.657	0.358	**8.3 (2.1)**
Crop*Weeds*Stage	0.900	0.718	0.625	0.122	0.536	0.135	**7.5 (3.4)**

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