



## Interactive effects of non-fodder litter and fungal species on soil enzymes: A microcosm temporal assessment from Indian arid zone

**Manohar Singh Suthar**

Department of Botany, Jai Naryan Vyas University, Jodhpur, Rajasthan, India.

**Manish Mathur** ✉

ICAR-Central Arid Zone Research Institute, Jodhpur, India.

**Praveen Gehlot**

Department of Botany, Jai Naryan Vyas University, Jodhpur, Rajasthan, India.

**Swami Sundaramoorthy**

Department of Botany, Jai Naryan Vyas University, Jodhpur, Rajasthan, India.

ARTICLE INFO	ABSTRACT
<p>Received : 27 November 2022 Revised : 17 January 2023 Accepted : 22 January 2023</p> <p>Available online: 13 April 2023</p> <p><b>Key Words:</b> Indian arid region Plant litter <i>Aspergillus</i> Soil enzymes Soil quality index</p>	<p>The interactive effects of three non-fodder Indian arid plant species, <i>Tephrosia purpurea</i>, <i>Aerva persica</i>, and <i>Calligonum procera</i>, and four <i>Aspergillus</i> fungal species on soil enzymes (acid and alkaline phosphatase, <math>\beta</math>-glucosidase, dehydrogenase, urease, and amidase activities) were temporally assessed (15 and 30 days withdrawal). The results were statistically analysed using ANOVA, Principal Component Analysis (PCA), and Canonical Correlation Analysis (CCoA). Aside from these, a biochemical soil quality index was created by assigning a weighted score to each enzyme and analysing it using PCA. This study found that various litter-fungal species complexes acted differently and that their effects changed over time, specifically for acid phosphatase, alkaline phosphatase, <math>\beta</math>-glucosidase, and amidase. Dehydrogenase and urease activities increased with predictors over time. With temporal backwash, all four fungal species with <i>C. procera</i> inhibit acid phosphatase, alkaline phosphatase, and <math>\beta</math>-glucosidase activities (i.e., more at 15 days and lesser after 30 days). Our current findings suggest that (a) urease activities were modulated by <i>A. persica</i> in cooperation with fungi like <i>A. terreus</i>, <i>A. niger</i>, and <i>A. flavus</i> at specific enzyme levels; (b) In assistance with fungi such as <i>A. fumigatus</i>, <i>A. niger</i>, and <i>A. persica</i>, amidase concentration was successfully managed through litter of the legume plant species <i>T. purpurea</i>. (c) When <i>C. procera</i> and <i>A. fumigatus</i>, <i>A. niger</i>, and <i>A. flavus</i> worked together, they were most effective at supporting <math>\beta</math>-glucosidase and dehydrogenase (d) Alkaline phosphatase and (e) acid phosphatase was more responsive to <i>T. purpurea</i>-<i>A. terreus</i> complexes than were <i>T. purpurea</i>-<i>A. flavus</i> and <i>C. procera</i>-<i>A. terreus</i> complexes.</p>

### Introduction

Soil health is the net result of ongoing conservation and degradation processes; which is heavily dependent on the biological elements of the soil ecosystem and affects plant health, environmental health, food safety, and quality (Laishram *et al.*, 2012). Numerous indicators can be used to gauge this, but soil enzymes are among the most significant because they are essential for preserving soil fertility, soil health, and soil ecology (Mathur and Sundaramoorthy, 2019). They react quickly to

changes in environmental circumstances and soil management practices. Because of this, they are used as sensors, and they have been researched all over the world as a measure of soil fertility (Utobo & Tewari, 2015), a measure of microbial biomass (Ren *et al.*, 2018), as indicators of vegetation effects and capability to conduct bio-geochemical cycling, total microbial activity (Luo *et al.*, 2018), as a predictor of bio-remediation (Basak *et al.*, 2016), and to understand the consequence of

degradation (Pajares *et al.*, 2011; Samuel, 2017). Their responses have also been assessed in terms of litter type (Xiang *et al.*, 2018; Jagadish *et al.*, 2001), soil fungi (Oseni, 2011), soil management practises (Maharjan *et al.*, 2017), and temporal variations (Ren *et al.*, 2018; Veeraragavan *et al.*, 2018). Some of the above attributes have been addressed in Indian arid and semi-arid zones by Tarafdar *et al.* (2002) and Gaur *et al.* (2012).

Few studies have been done on the interactions between different types of plant litter and different fungi species and extracellular soil enzymes in hot dry region of India. In light of this, our study closes a knowledge gap regarding the impact of fungus species and litter type on extracellular soil enzymes. We hypothesized in this work that the effects of particular plant litter and fungus species on soil enzymes would vary from their combined effects and that these variances would change over time. The objectives of this study were (a) to evaluate the temporal effects of litter from three wild arid plant species on acid phosphatase, alkaline phosphatase, beta-glucosidase, dehydrogenase, urease, and amidase, i.e. after 15 and 30 days withdrawal periods. (b) to visualise the studied enzymes in a cumulative approach that takes into account the interactive effects of the litter-fungi-time complex, and (c) to develop a biochemical soil quality index.

## Material and Methods

### Species and experimental setup

As litter source species, three non-fodder arid wild plant species were used: *Tephrosia purpurea* (L.) Pers, *Aerva persica* (Burm.f.) Shult and *Calotropis procera* (Aiton) W.T.Aiton. These species can be found in abundance in wastelands, fallow lands, crop-fences, and community grazing lands (Mathur and Pandey, 2010). As litter, total aboveground biomass of *T. purpurea* and *A. persica*, as well as a mixture of stem (1 cm width) and leaves of *C. procera*, were used. These species were collected in and around natural agroforestry field located at six different provinces of arid eco-regions, namely Shergarh (Site 1; 26° 19' 32.66" N and 72° 17' 13.16" E), Balesar (Site 2; 26° 24.0' 69" N and 72° 28' 59.83" E), Osijan (Site 3; 26° 43' 53.46" N and 72° 54' 30.79" E), Chawa (Site 4; 26° 22.06' 62" N and 72° 09.05' 58" E), Baitu (Site 5; 25° 54' 13.72" N and 71° 46' 15.69" E) and Kalyanpur (Site 6; 26°

0.1' 19.67" N and 72° 34' 34.21" E). After being collected, each plant material was air dried in comparable climatic conditions. After achieving a homogeneous moisture level, they were completely blended to create homogeneous material. For a microcosm experiment, four different fungi—*Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, and *Aspergillus terreus*—were chosen and inoculated with plant litter from non-fodder plants. The plant pathology lab of the Department of Botany at Jai Narain Vyas University in Jodhpur, India, provided these fungal cultures. The same department's experimental field of ecology laboratory provided the soil, which was then pulverized and sieved through a 2 mm mesh size in preparation for the microcosm experiment. Then, over the course of three days, this natural soil was tyndalized by autoclaving at 121° C for an hour and overnight oven drying at 80° C. (Eivazi and Tabatabai, 1982).

100% tyndalized soil was placed in 250ml cotton plugged conical flasks, followed by litter at a fertility level of 2000 kg N h<sup>-1</sup> and the fungal culture (on the basis of colony forming unit 3 X 10<sup>9</sup> per treatment). After 15 and 30 days of laboratory incubation, this microcosm experiment setup was recovered, and six different extracellular enzymes were quantified. Acid and alkaline phosphatase (Eivazi and Tabatabai, 1977), glucosidase (Eivazi and Tabatabai 1988), dehydrogenase (Tabatabai, 1982), urease (Douglas and Bremner, 1977a and b), and amidase (Frankenberger and Tabatabai, 1980) were all quantified using standard methodologies. After incubation and enzyme reaction termination, each enzyme activity was kept under control by adding substrate to blank samples (Mathur, 2005). All the experiments were conducted in triplicates.

### Statistical analysis

To analyze the effects of three major sources of variation (litter types, fungal species, and withdrawal time) and their interactions on the concentration of the examined enzymatic activities, we utilized Statsoft's (2011 Version 10) three-way ANOVA (Strip-Split design) tool. The intersection plot is divided into subplots in the strip-split-plot design to accommodate a third element, extending the capabilities of the strip-plot design. This design stands out due to the use of four levels of precision for measuring the effects of various factors, with the highest level corresponding to the sub-plot

factor and its interactions with other factors, as well as more than three plot sizes (such as horizontal, vertical strip, intersection plot, and subplot). The percentage temporal deviation in various soil enzymes was calculated in relation to litter type and fungal species by using the following formula.

$$\% \text{ Temporal Deviation} = \left( \frac{W_2 - W_1}{W_1} \right) \times 100 \quad (1)$$

Where  $W_1$  and  $W_2$  are first and second withdrawal periods. In this study, principal component analysis (PCA) was used for two distinct purposes: (a) to visualize the litter-fungal complex with reference to their proximity or distance with withdrawal periods for each studied enzyme; and (b) to develop a soil quality index with weighted scores assigned to each enzyme. The distance of each litter-fungal complex from the centroid of the PCA bi-plot developed in earlier steps was calculated. A cumulative data set that includes the litter-fungi complex for each enzyme under consideration was produced using these centroid distances. This cumulative data set was used to create the PCA bi-plot, which graphically depicts the overall scenario for various enzymes that is naturally adjusted by litter-fungal withdrawal as well as their interactions. Such PCA strategies have been supported by many researchers (Laliberte and Legendre, 2010, Mathur and Sundaramoorthy, 2018). The connections between soil enzymes detected during the first and second withdrawal periods, as well as between these two periods, were established using Canonical Correlation Analysis (CCoA). Correlation matrices were converted into a network-like structure for ease of graphical interface. PCA was used to quantify correlations among enzymes with weighted scores using the FAST (Hammar *et al.* 2001) and XLSTAT (2017) software.

Weights in the soil quality index were determined by dividing the percent of variation in the data set explained by the principal component analysis that contributed the indicated variable by the total percentage of variance explained by all the PCs with more than one eigenvector (Mathur and Sundaramoorthy, 2018).

$$SQI = \sum_{i=1}^n W_i \times S_i \quad (2)$$

$W_i$  = weighting factor of soil enzyme  $i$  and  $S_i$  value of soil enzyme  $i$ .

## Results and Discussion

### Acid phosphatase ( $\mu\text{g p-Nitrophenol released h}^{-1} \text{g}^{-1}$ of soil)

This enzyme had a higher concentration (0.97) with *C. procera* + *A. terreus* after 15 days, but it had a lower concentration (0.65) after 30 days with a different plant-fungal complex (i.e., *A. persica* + *A. niger* Table 1). Except for *A. persica* + *A. niger* and *T. purpurea* + *A. fumigatus*, we found higher concentrations of this enzyme during 15 days withdrawal compared to 30 days with most treatments. We noted +4.40 and +5.41 percent temporal deviation with these two complexes. (Table 2).

### Alkaline phosphatase ( $\mu\text{g p-Nitrophenol released h}^{-1} \text{g}^{-1}$ of soil)

Higher concentrations were found in *C. procera* + *A. fumigatus* (1.37) and *C. procera* + *A. terreus* (1.50), respectively, at 15 and 30 day intervals (Table 1). While its minimum concentration was recorded at 15 and 30 day intervals with *A. persica* + *A. niger* (0.58) and *A. persica* + *A. fumigatus* (0.87), respectively. With the exception of *C. procera* and *T. purpurea* + *A. terreus*, all four fungal species showed negative temporal deviation (Table 2), indicating that they were recorded more during the first sampling period.

### Beta-glucosidase ( $\mu\text{g p-Nitrophenol released h}^{-1} \text{g}^{-1}$ of soil)

During the two sampling periods, higher concentrations of this enzyme (1.19 and 1.14) were detected with the *C. procera* + *A. fumigatus* complex (Table 1). Its minimum concentrations with *T. purpurea* + *A. flavus* (0.35 at 15 days) and *T. purpurea* + *A. fumigatus* (0.35 at 15 days) were determined (0.42 at 30 days). Similar to Alkaline Phosphatase, all four fungal species with *C. procera* and *T. purpurea* + *A. fumigatus* exhibited negative temporal deviations (Table 2).

### Dehydrogenase ( $\mu\text{g TPF released h}^{-1} \text{g}^{-1}$ of soil)

*Calotropis procera* was found to be more conducive for this enzyme with four studied fungi, with higher concentrations recorded with *C. procera*-*A. flavus* (12.68) and *C. procera*-*A. fumigatus* (12.67) during second sampling periods (i.e., 30 day). During the first sampling period (15

**Table 1: Effects of soil fungi and litter types on various soil enzymes during two sampling times.**

Variables	AcP		AIP		BG		De		Ur		Am	
	1	2	1	2	1	2	1	2	1	2	1	2
Control	0.39	0.23	0.6	0.46	0.16	0.34	1.74	1.57	164.51	158.24	0.16	0.17
T. purpurea+ A. flavus	0.64	0.54	1.11	1.16	0.35	0.64	3.54	5.33	193.72	286.92	0.25	0.27
T. purpurea+ A. fumigatus	0.47	0.50	0.61	0.91	0.49	0.42	1.84	4.44	222.47	257.71	0.24	0.12
T. purpurea+ A. niger	0.59	0.44	1.07	1.21	0.42	0.43	1.78	8.72	240.76	277.42	0.25	0.08
T. purpurea+ A. terreus	0.64	0.63	1.25	1.21	0.39	0.56	1.54	10.51	249.61	275.81	0.25	0.40
A. persica +A. flavus	0.67	0.44	0.98	1.03	0.40	0.69	1.72	8.12	240.38	338.53	0.24	0.26
A. persica +A. fumigatus	0.59	0.50	0.62	0.87	0.52	0.68	2.01	2.35	240.88	272.76	0.27	0.09
A. persica +A. niger	0.61	0.65	0.58	1.16	0.52	0.87	2.35	10.05	231.31	406.45	0.26	0.24
A. persica +A. terreus	0.72	0.60	0.62	1.17	0.56	0.74	2.51	10.11	254.67	365.23	0.28	0.32
C. procera +A. flavus	0.73	0.50	1.50	0.89	0.97	0.69	3.15	12.68	186.87	293.55	0.34	0.16
C. procera +A. fumigatus	0.88	0.47	1.87	1.12	1.19	1.14	4.49	12.67	235.45	330.11	0.32	0.18
C. procera +A. niger	0.91	0.54	1.70	0.90	1.08	0.71	5.84	10.90	214.09	305.38	0.36	0.13
C. procera +A. terreus	0.97	0.53	1.40	1.38	0.98	0.78	7.52	12.26	236.60	346.77	0.32	0.35

AcP = Acid Phosphatase, AIP = Alkaline Phosphatase, BG = Beta-glucosidase, De = Dehydrogenase, Ur = Urease, Am = Amidase. 1 and 2 are = 15 and 30<sup>th</sup> days withdrawal, respectively.

**Table 2: Percent temporal deviation in various soil enzymes with Litter types and fungal species**

Variables	AcP	AIP	BG	De	Ur	Am
Control	-40.19	-22.57	109.13	-12.91	-3.81	5.14
T. purpurea+ A. flavus	-15.81	5.39	80.16	50.66	48.11	6.34
T. purpurea+ A. fumigatus	5.41	48.77	-14.52	140.64	27.28	-51.16
T. purpurea+ A. niger	-26.40	13.65	2.43	390.87	15.23	-67.68
T. purpurea+ A. terreus	-1.96	2.95	43.05	580.23	10.49	63.02
A. persica +A. flavus	-33.30	4.45	75.09	372.13	40.83	10.77
A. persica +A. fumigatus	-15.72	40.42	30.50	390.71	13.23	-65.46
A. persica +A. niger	7.40	100.09	67.42	287.11	75.71	-8.52
A. persica +A. terreus	-16.57	89.30	31.32	302.87	43.42	11.16
C. procera +A. flavus	-31.22	-40.57	-29.18	228.40	57.09	-53.16
C. procera +A. fumigatus	-46.11	-39.97	-4.75	182.30	40.21	-45.31
C. procera +A. niger	-40.82	-47.05	-30.02	86.68	42.64	-62.56
C. procera +A. terreus	-45.55	-1.80	-20.83	63.06	46.57	9.48

AcP = Acid Phosphatase, AIP = Alkaline Phosphatase, BG = Beta-glucosidase, De = Dehydrogenase, Ur = Urease, Am = Amidase

days), the concentration of this enzyme was lower with *T. purpurea* - *A. terreus* (1.54) and with *A. persica*-*A. flavus*, *T. purpurea* - *A. niger*, and *T. purpurea*-*A. fumigatus* (Table 1). *T. purpurea*-*A. terreus* had the highest percent positive deviation (580.23) over two sampling periods (Table 2). Such findings imply that the efficacy of this litter-fungi complex is time-dependent.

#### Urease ( $\mu\text{g}$ urea hydrolyzed released $\text{h}^{-1}\text{g}^{-1}$ of soil)

The effects of litter type and fungal species on this enzyme were more pronounced during the second sampling period (Table 1). The highest concentration (406.45) was obtained with *A. persica*-*A. niger*, while the complex of *C. procera*-*A. flavus* after 15 days withdrawal was the least effective (186.87) for this enzyme, and the highest positive deviation (75.82) was obtained with *A. persica*-*A. niger* (Table 2).

#### Amidase ( $\mu\text{g}$ $\text{NH}_4^+$ released $\text{h}^{-1}\text{g}^{-1}$ of soil)

The effects of litter types and soil fungi on the concentration of this enzyme were greatest (0.40) with *T. purpurea* -*A. terreus*, but the least (0.08) with the same litter type but with *A. niger* (Table 1). Impacts of *C. procera* with different soil fungi were recorded more during 15 days withdrawal compared to 30 days. Both positive and negative temporal deviations were recorded for this enzyme and *T. purpurea* - *A. flavus*, *T. purpurea*-*A. terreus*, *A. persica*-*A. terreus*, *C. procera*-*A. terreus* supports concentration of this enzyme with time advancement. However, effect of *T. purpurea*-*A. niger*, *T. purpurea*-*A. fumigatus*, *A. persica*-*A. flavus*, *A. persica*-*A. niger*, and *C. procera* with *A. flavus*, *A. fumigatus*, *A. niger* on enzyme concentration held back with time advancement (Table 2). The results of the analysis of variance (ANOVA) revealed that all of the studied variables, including litter type, fungal species, and sampling time, as well as their interactions, caused significant variations in alkaline phosphatase, dehydrogenase, and urease enzymes. Significant differences in acid phosphatase were brought about by litter and fungal species, as well as sampling time. Their interactions, however, were insignificant. For beta-glucosidase, neither the sampling factor nor its

interaction with fungal species were significant. For amidase, litter types and their interactions with sampling time were not significant. The effects of predictors on enzyme activities are clearly visible as we have got many fold increase in concentration of studied enzyme (Table 1) in comparison to control. However, treatments like *T. purpurea*-*A. terreus* (15 days) for dehydrogenase (-10.70%) and *T. purpurea*-*A. fumigatus* (-25.47%), *T. purpurea* - *A. niger* (-49.74%), *A. persica*-*A. fumigatus* (-41.07%) and *C. procera*-*A. niger* (-16.81%) for amidase (30 days) revealed their negative impacts on these enzymes with compared to control. Bi-plots of all the soil enzymes showed that first two axes (F1 and F2) together accounted 100 per cent variabilities (60.41 and 39.58, 64.06 and 35.79, 86.11 and 13.88, 75.86 and 24.12, 81.96 and 18.03, 51.60 and 48.39) for acid phosphatase, alkaline phosphatase, beta-glucosidase, dehydrogenase, urease and amidase, respectively). Such results indicated the appropriate use of this tool as the cumulative percentage of variance for each enzyme approached >80 per cent. Certain specific trends were visualized that were pertains to litter-fungal complex and with withdrawal time (a) *C. procera* with *A. fumigatus*, *A. niger* and with *A. flavus*, was more conducive for acid phosphatase, alkaline phosphatase, beta-glucosidase and for amidase with 15 days period, (b) *A. niger* and *A. terreus* with litter like *T. purpurea*, *A. persica* and *A. fumigatus*, *A. terreus* with *A. persica* and with *C. procera* were more effective for dehydrogenase enzyme with 30 days, while *C. procera* with *A. niger* and with *A. terreus* having an intermediate temporal positive impact for this enzyme (c) *T. purpurea* with *A. fumigatus*, *A. niger*, *A. terreus* showed a temporal progressive pace for urease and a decreased temporal pace were showed by *T. purpurea* with *A. fumigatus*, *niger* and with *A. persica* - *A. fumigatus* for amidase enzyme. Further with these PCA analysis, the factorial score (distance from centroid) for each litter-fungi complexes along with control and for enzymes were calculated and are presented in (Table 3). With this data set a bi-plot was further constructed (Figure 1) representing all the studied enzymes and litter-fungal complex along with accommodated withdrawal period.

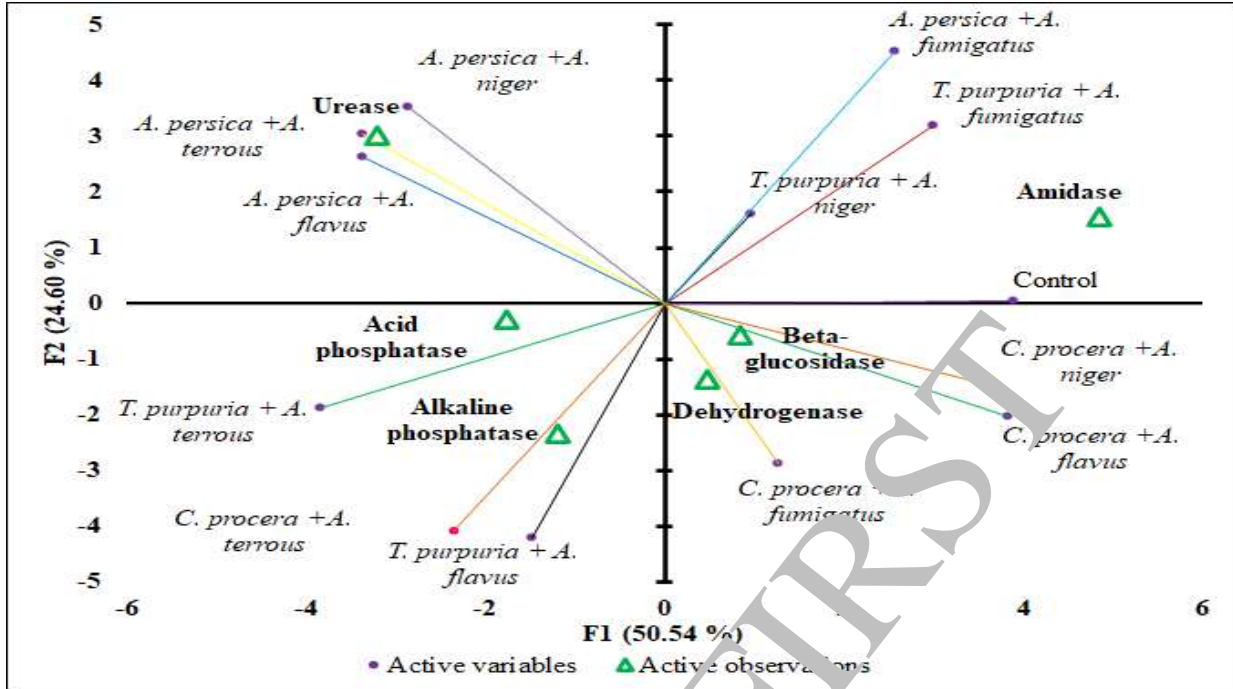


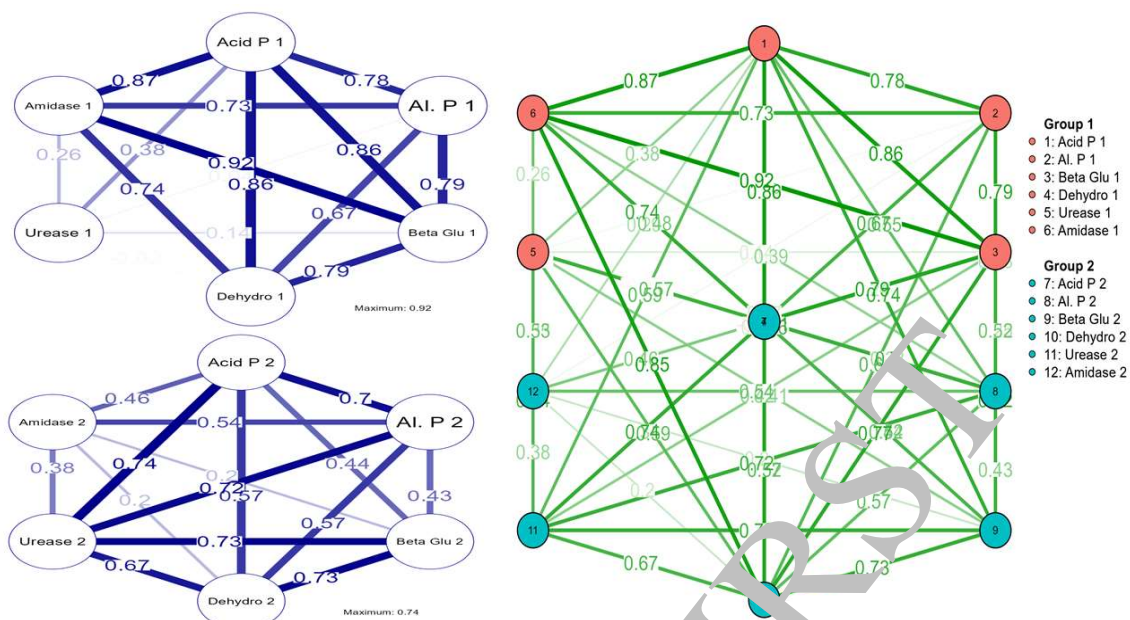
Figure 1: PCA Bi-plot with Factorial Score of Different Enzymes with different treatments.

For this the per cent variability explained by first four axes were, 50.54, 24.60, 13.15 and 7.7 with their eigen value, 6.5, 3.19, 1.7 and 1.0 respectively. This approach suggested a more cumulative relationship among enzyme-litter-fungi complex and revealed that (a) *A. persica* and fungi like *A. terreus*, *A. niger*, *A. flavus* are crucial one for the urease (b) *T. purpurea* with *A. fumigatus* and *A. niger* and *A. persica*- *A. fumigatus* were more effective for amidase, (c) *C. procera* with *A. fumigatus*, *A. niger* and with *A. flavus* were more supportive for beta-glucosidase and dehydrogenase, (d) *C. procera*-*A. terreus* and *T. purpurea*- *A. flavus* found to be effective with alkaline phosphatase and (e) Acid phosphatase having more proximity with *T. purpurea* -*A. terreus*. With this tool our biochemical soil quality equation was developed which can be equate as

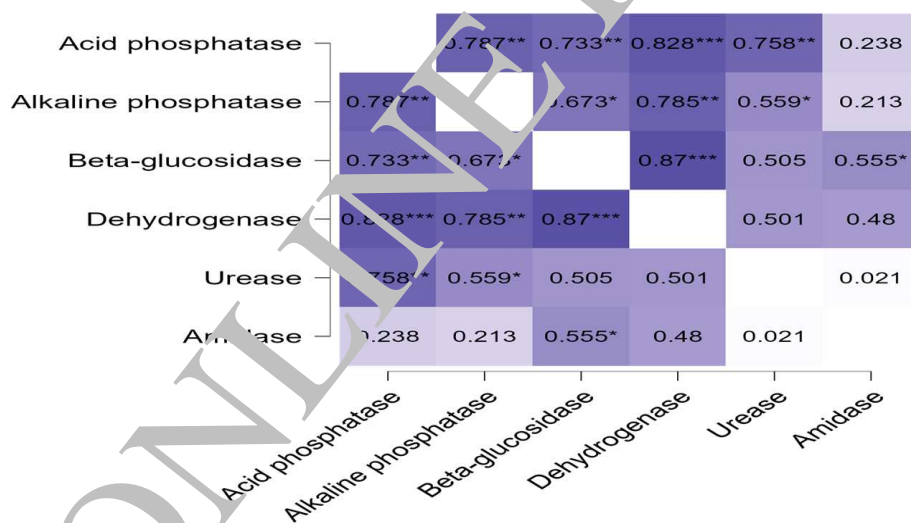
$$SQI = \sum_{i=1}^n - 0.68 \text{ Acid Phosphatase} - 0.46 \text{ Alkaline Phosphatase} + 0.32 \text{ Beta-glucosidase} + 0.18 \text{ Dehydrogenase} + 1.89 \text{ Amidase} - 1.25 \text{ Urease} \quad (3)$$

Here, the numbers represent the distance of each enzyme from the centroid and sign represent their relative position on PCA bi-plot (Figure 1). Use of

PCA in the development of a soil quality index are provided by many workers also (Laishram et al., 2012; Cherubin et al., 2016; Guo et al., 2018). Significant correlations among enzymes (studied during first and second withdrawal and between enzymes quantified during these two sampling periods and among enzymes) are depicted in Figure 2. CCoA revealed that urease during the first withdrawal period not related to any other studied enzymes, however, it showed correlation with almost all enzymes during second sampling period except amidase. Significant intra-relationship was also recorded between BG<sub>1</sub> and BG<sub>2</sub> (r<sup>2</sup> = 0.72). Interestingly, amidase during the first sampling period was significantly correlated with all studied enzymes except urease, however, during the second sampling period, it was linked with alkaline phosphatase (ALP<sub>2</sub>) only (r<sup>2</sup> = 0.54). Correlations among weighted enzymes (Figure 3) suggested that both acid phosphatase as well as alkaline phosphatase related to all other enzymes except amidase. Amidase (r<sup>2</sup> = 0.55) and urease (r<sup>2</sup> = 0.56) were correlated with beta-glucosidase and with alkaline phosphatase, respectively. The strongest relationships were presented between BG<sub>1</sub>-Am<sub>1</sub> (r<sup>2</sup> = 0.92) and between BG<sub>w</sub> - De<sub>w</sub> (r<sup>2</sup> = 0.87).



**Figure 2: Correlation matrix for different soil enzymes (temporal, and combined)**  
 Significant relationships are indicated by bold lines). Acid P = Acid Phosphatase; Al P = Alkaline Phosphatase; Beta Glu = Beta-glucosidase; Dehydro = Dehydrogenase. 1, 2, represents the 15<sup>th</sup> and 30<sup>th</sup> days withdrawals.



**Figure 3: Correlation matrix for different soil enzymes (weighted)**

Extracellular enzyme activities (EEAs) are indicators of both soil microbial activity and nutrient availability for plants. However, it is unclear how EEAs change in response to litter and micro-organism inputs particularly in the Indian hot desert. The effects of the desertic environment on soil enzymes have been explored with different predictors like plantation regime of *Caragana microphylla* (Cao *et al.* 2008), soil properties

(Stursova and Sinsabaugh, 2008; Buscardo *et al.* 2021), biological soil crust (Liu *et al.* 2014), effects of rainfall treatments (Laura *et al.* 2015), long term restoration of desertified land (Zhang *et al.* 2015a), effects of grazing and cultivation (He *et al.* 2017). The temporal dynamics of enzyme activities should reflect the availability of substrates (Zhang *et al.* 2015b). Our findings address this discrepancy by indicating that soil enzyme activities vary with litter

**Table 3. Factorial Score for different enzymes and treatments calculated with PCA**

Variables	AcP	AIP	BG	De	Ur	Am
Control	-2.69	-2.27	-1.68	-1.78	-2.5	-1.23
<i>T. purpurea</i> + <i>A. flavus</i>	0.09	0.4	-0.54	-0.52	-0.73	-0.68
<i>T. purpurea</i> + <i>A. fumigatus</i>	-0.81	-1.02	-0.88	-1.23	-0.82	0.27
<i>T. purpurea</i> + <i>A. niger</i>	-0.73	0.48	-0.99	-0.49	0.15	0.62
<i>T. purpurea</i> + <i>A. terreus</i>	0.56	0.74	-0.68	-0.25	0.32	-1.7
<i>A. persica</i> + <i>A. flavus</i>	-0.4	-0.15	-0.33	-0.6	0.72	-0.85
<i>A. persica</i> + <i>A. fumigatus</i>	-0.36	-1.1	-0.15	-0.22	0.11	0.85
<i>A. persica</i> + <i>A. niger</i>	0.62	-0.36	0.35	0	1.12	-0.32
<i>A. persica</i> + <i>A. terreus</i>	0.73	-0.29	0.08	-0.01	1.27	-0.57
<i>C. procera</i> + <i>A. flavus</i>	0.15	0.2	0.65	0.87	0.81	1.33
<i>C. procera</i> + <i>A. fumigatus</i>	0.52	1.39	2.24	1.07	0.54	0.96
<i>C. procera</i> + <i>A. niger</i>	1.04	0.52	1.02	1.1	-0.13	1.7
<i>C. procera</i> + <i>A. terreus</i>	1.26	1.43	0.92	1.98	0.72	-0.35

AcP = Acid Phosphatase, AIP = Alkaline Phosphatase, BG = Beta-glucosidase, De = Dehydrogenase, Ur = Urease, Am = Amidase

and fungal species types, as well as their combined actions. In this study, the temporal increase in enzyme activities such as dehydrogenase and urease could be attributed to litter's dual role in (a) providing suitable substrate for microbial activities and (b) contributing to the formation and stability of soil aggregates. These findings are consistent with those of Acosta-Martinez *et al.* (2002) and Fang *et al.* (2013). Using saline plant species like *Asteriscus maritimus*, *Arthrocnemum macrostachyum*, *Frankenia corymbosa*, *Halimione portulacoides*, *Limonium cossonianum*, *Limonium caesium*, *Lygeum spartum*, and *Suaeda vera*, Caravaca *et al.* (2005) reported significant variations in dehydrogenase, urease, phosphatase, and beta-glucosidase. They also came to the conclusion that this alteration may be attributable to various microbial communities connected to the rhizosphere soil or to various quantities of microbial biomass carbon. Effects of monoculture and polyculture practices on rhizosphere enzyme activities were studied by Yang *et al.* (2007), Fang *et al.* (2013) and Bogat and Walczak (2022). They concluded that cause-effect relationships between type of cultural practices and modification in rhizosphere enzyme concentration cannot be generalized and they are region and species specific. The seasonal dynamics of soil enzyme activities in response to leaf litter of *Cassia siamea*, *Shorea robusta*, *Eucalyptus citriodora*, *Acacia auriculiformis*, *Anacardium occidentale*, *Dalbergia sissoo* was reported by Venu *et al.* (2016). Two

fundamental lines of thought can be contributed in light of the present findings: (a) different litter-fungal species complexes functioned differently at the level of the specific enzyme. Particularly for beta-glucosidase, amidase, acid phosphatase, and alkaline phosphatase, their effects evolved with time. The aforementioned factors led to an increase in dehydrogenase and urease activity over time. All four fungi containing *C. procera* suppress the activities of acid phosphatase, alkaline phosphatase, and beta-glucosidase with temporal backwash (i.e., more at 15 days and lesser after 30 days). Such patterns were seen for amidase except with *C. procera*-*A. terreus*. As a result of this study, a litter-fungi species specific complex can be recommended for the studied enzyme, (b) all enzyme predictors: We created a bio-chemical soil quality index using PCA and CCoA, in which each enzyme was weighted with numerical values based on their relationships with litter type (plant species), fungi species types, and withdrawal period (temporal factor). As a result, faith in studied enzymes with litter-fungi-time complex is provided. At specific enzyme levels, our current findings suggest that (a) urease activities were modulated by *Aerva persica* in collaboration with fungi such as *A. terreus*, *A. niger*, and *A. flavus* (b) amidase concentration was effectively controlled through litter of a legume plant species *T. purpuria* in collaboration with fungi such as *A. fumigatus*, *A. niger*, and *A. persica* (c) Beta-glucosidase and dehydrogenase were most supportive by combined



action of *C. procera* with *A. fumigatus*, *A. niger*, *A. flavus* (d) *C. procera*-*A. terreus* and *T. purpurea*-*A. flavus* complexes, on the other hand, were more effective for alkaline phosphatase and (e) acid phosphatase having more proximity with *T. purpurea*-*A. terreus*. Our correlation analysis revealed the complex relationships between enzymes using an enzyme-specific approach. Canonical Correlation Analysis (CCoA) revealed that urease did not correlate with any other enzymes studied during the first withdrawal period, but it did correlate with almost all enzymes except amidase during the second sampling period. Surprisingly, amidase was significantly correlated with all studied enzymes except urease during the first sampling period, but only alkaline phosphatase (AIP<sub>2</sub>  $r^2 = 0.54$ ) during the second sampling period. Correlations among weighted enzymes revealed that acid phosphatase and alkaline phosphatase were both related to all other enzymes except amidase. These findings are consistent with those of Acosta-Martinez *et al.* (2003) and Mathur (2020). The region's first compressive effort employing locally accessible desert plant species and fungus resulted in the development of a soil quality index. This index considers how time, litter, and different fungal species behave. This index, however, may not be applicable to other scenarios such as cultural practices (addition of fertilizers, irrigation types, soil tillage, fallow land practice, and so on), land use (forest land, agricultural land, orchid, and so on), mining, and rehabilitation practices because the studied dataset is restricted to controlled conditions. Such practices unquestionably have a unique interaction with the biochemical properties of soil. Future work will therefore involve validating and improving this index using numerous scenarios.

## Conclusion

The current study is the first from the hot, arid region of India to look at the interaction and temporal effects of diverse fungus species and wild plant species used as litter sources on soil enzyme concentrations. Using PCA and CCoA, we developed a bio-chemical soil quality index, where each enzyme was weighted with numerical values based on their associations with the kind of litter (plant species), fungal species types, and

withdrawal period (temporal factor). The idea of sustainable utilization of arid wild species that are neither cultivated nor have direct market potential can be used to determine the importance of the current study (provisional ecosystem services). They can therefore be utilized as regular litter for a variety of crops to maintain soil fertility.

## Acknowledgement

The authors are thankful to the Head, Department of Botany for facilities, IGC-CAS and DST-FIST are acknowledged for instrumental facilities. The corresponding author is thankful to the Director, CAZRI for granting study leave during the research period.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

- Acosta-Martinez, V., Zobeck, T.M., Gill, T.E., & Kennedy, A.C. (2003) Enzyme activities and microbial community structure in semiarid agricultural soils. *Biology and Fertility of Soils*, 38, 216-227.
- Basak, B., & Dey, A. (2016) Bioremediation approaches for recalcitrant pollutants: potentiality, successes and limitation. In: Ashok KR, Vindo KD, Editors. Toxicity and Waste Management Using Bioremediation. IGI Global Engineer Science, Series Advances in Environmental Engineering and Green Technologies. Hershey PA, USA. 178-197.
- Bogati, K., & Walczak, M. (2022) The Impact of Drought Stress on Soil Microbial Community, Enzyme Activities and Plants. *Agronomy*, 12, 189. <https://doi.org/10.3390/agronomy12010189>
- Buscardo, E., Souza, R.C., Meir, P., Gemi, J., Schmidt, S.K., da Costa, A.C.L. & Nagy, L. (2021). Effects of natural and experimental drought on soil fungi and biogeochemistry in an Amazon rain forest. *Communications Earth and Environment* 2, 55 <https://doi.org/10.1038/s43247-021-00124-8>
- Cao, C., Jian, D., Teng, X., Jiang, Y., Liang, W., & Cui, Z. (2008) Soil chemical and microbiological properties along a chronosequence of *Caragana microphylla* Lam. Plantations in the Horqin sandy land of northeast china. *Applied Soil Ecology*, 40, 78-85.
- Caravaca, F., Alguacil, M.M., Torres, P., & Roldan, A. (2005) Plant type mediates rhizospheric microbial activities and

- soil aggregation in a semiarid Mediterranean salt marsh. *Geoderma*, 124, 375-382.
- Cherubin, M.R., Karlen, D.L., Cerri, C.E.P., Franco, A.L.C., Tormena, C.A., & Davies, C.A. (2016) Soil Quality Indexing Strategies for Evaluating Sugarcane Expansion in Brazil. *PLoS ONE*, 11(3), 1-26.
- Douglas, L.A., & Bremner, J.M. (1970b). Colorimetric determination of microgram quantities of urea. *Analytical Letters*, 3,79-87.
- Douglas, L.A., & Bremner, J.M. (1970a). Extraction and colorimetric determination of urea in soils. *Soil Science Society of America Proceeding: Soil Science*, 859-862.
- Eivazi, F., & Tabatabai, M.A. (1977) Phosphatases in soils. *Soil Biology and Biochemistry* 9, 167-172.
- Eivazi, F., & Tabatabai, M.A. (1988) Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, 20(5), 601-606.
- Fang, S., Liu, D., Tian, Y., Deng, S., & Shang, X. (2013) Tree Species Composition Influences Enzyme Activities and Microbial Biomass in the Rhizosphere: A Rhizobox Approach. *PLoS ONE*, 8(4),1-11
- Frankenberger, JrW.T., & Tabatabai, M.A. (1980) Amidase activity in soils: Method of Assay. *Soil Science Society of America Journal*, 44, 282-287.
- Gaur, D., Jain, P.K., & Bajpai, V. (2012). Production of extracellular  $\alpha$  amylase by thermophilic *Bacillus* sp. isolated from arid and semi-arid region of the Rajasthan, India. *Journal of Microbiology and Biotechnology Research*, 2(5), 675-684.
- Guo X.M., Tong-qian, Z., Wen-ke, C., Chun-yan, X., & Yu-xiao, H. (2018) Evaluating the effect of coal mining subsidence on the agricultural soil quality using principal component analysis. *Chilean Journal of Agricultural Research*, 78(2),173-182.
- Hammar, O., Harper, D.A.T., & Ryan. P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaentologica Electronica*, 4 (1), 9pp.
- Jagadish, C.T., Subhash, C.M., & Shyam, K. (2001) Influence of straw size on activity and biomass of soil microorganisms during decomposition. *European Journal of Soil Biology*, 37,157-160.
- Laishram, J., Saxcena, K.G., Maikhuri, R.K., & Rao, K.S. (2012). Soil quality and soil health: a review. *International Journal of Ecology and Environmental Sciences*, 38(1), 19-37.
- Laliberte, E., & Legendre, P. (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 9(1), 299-305.
- Laura, M.L., Sinsabaugh, R.L., Collins, S.L., & Thomey, M.L. (2015) Soil enzyme responses to varying rainfall regimes in chihuahuan desert soils. *Ecosphere*, 6(3), 40.1-10
- Liu, Y., Yang, H., Li, X., & Xing, Z. (2014) Effects of biological soil crust on soil enzymes activities in revegetated areas of the tengger desert, China. *Applied Soil Ecology*, 80, 6-14.
- Luo, L., Meng, H., & Gu, J. (2018). Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, 175:539-549.
- Maharjan, M., Sanaullah, M., Khatwani, B.S., & Kuzykov, Y. (2017). Effect of land use and management practices on microbial biomass and enzyme activities in subtropical top- and sub-soils. *Applied Soil Ecology*, 113:22-28.
- Mathur, M. (2005). Ecology and prospecting of some medicinal plants of aphrodisiac potential. Ph.D. thesis. Jai Narain Vastu University, Jodhpur, Rajasthan, India.
- Mathur, M. (2020). Compartments of arid grazing land plant diversity: a temporal assessment with bottom-up and top-down factors. *Range Management and Agroforestry*, 41 (2020), 200-208.
- Mathur, M., & Pandey, C.B. (2016). Vegetation ecology of hot arid and semi arid grazing lands of India. In: Gaur M, Pandey CB, Goyal RK (eds) Remote sensing for natural resources monitoring and management. Scientific Publishers, Jodhpur, pp 213-242
- Mathur, M., & Sundaramoorthy, S. (2009). Mineral Composition in *Corchorus depressus* at heterogeneous environmental conditions and their relationships with bottom-up, top-down, and plant metabolite factors. *Communications in Soil Science and Plant Analysis*, 40(13), 2028-2043.
- Mathur, M., Suthar, M.S., Gehlot, P., & Sundaramoorthy, S.S. (2019) Assessment of litter availability and its quality plasticity of four wild species of the Indian arid environment. *Tropical Ecology*, <https://doi.org/10.1007/s42965-019-00034-z>
- Mathur. M., & Sundaramoorthy, S. (2018) Appraisal of arid land status: a holistic assessment pertains to bio-physical indicators and ecosystem values. *Ecological Processes*, 7(41), 1-15.
- Oseni, O.A. (2011) Production of microbial protease from selected soil fungal isolates. *Nigerian Journal of Biotechnology*, 23, 28-34.
- Pajares, S., Gallardo, J.F., Masciandaro, G., Ceccanti, B., & Etchevers, J.D. (2011) Enzyme activity as an indicator of soil quality changes in degraded cultivated acrisols in the Mexican trans-volcanic belt. *Land Degradation and Development*, 22, 373-381.

- Ren, Q., Song, H., Yuan, Z., Ni, X., & Li, C. (2018). Changes in soil enzyme activities and microbial biomass after revegetation in the three gorges reservoir, China. *Forests*, 9, 249.
- Samuel, A. D., Brejea, R., Domuta, C., Bungau, S., Cenusa, N., & Tit, D. M. (2017). Enzymatic indicators of soil quality. *Journal of Environmental Protection and Ecology*, 18(3), 871-878.
- StatSoft, Inc. (2011) STATISTICA (Data Analysis Software System), Version 10. <http://www.statsoft.com>
- Stursova, M., & Sinsabaugh, R.L. (2008). Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. *Soil Biology and Biochemistry*, 40, 550-553.
- Tabatabai. M. A. (1982) Soil enzymes In: Page A.L., Miller R.H., Keeney D.R. (eds.): *Methods of Soil Analysis, Part 2*. American Society of Agronomy and Soil Science Society of America, Madison.
- Tarafdar, J.C., Yadav, R.S, & Niwas, R. (2002). Relative efficiency of fungal intra-and extracellular phosphatases and phytase. *Journal of Plant Nutrition and Soil Sciences*, 165, 17-19.
- Utobo, E.B., & Tewari, L. (2015). Soil enzymes as bio indicators of soil ecosystem status. *Applied Ecology and Environmental Sciences*, 13(1), 147-169.
- Veeraragavan, S., Duraisamy, R., & Mani, S. (2018) Seasonal variation of soil enzyme activities in relation to nutrient and carbon cycling in *Senna alata* (L.) Roxb invaded sites of Puducherry region, India. *Geology Ecology and Landscapes*, 2(3), 155-168.
- Venu, N., Reddy, S.V., & Reddy, M. (2016). Estimation of soil enzyme activity with respect to decomposition of leaf litter types. *Bioline*, 4 (1), 155-163.
- Xiang, Y., An, S., Cheng, M., Liu, L., & Xie, Y. (2018). Changes of soil microbiology properties during grass litter decomposition in loess hilly region, China. *International Journal of Environmental Research and Public Health*, 15, 1797.
- XLSTAT. 2017. *Data Analysis and Statistical Solution for Microsoft Excel*. Addinsoft, Paris, France.
- Yang, R., Tang, J., Chen, X., & Hu, S. (2007) Effects of coexisting plant species on soil microbes and soil enzymes in metal lead contaminated soils. *Applied Soil Ecology*, 37, 240-246.
- Zhang, S., Li, J., Wang, X., & Sun, B. (2015a). Effects of soil management regimes on biochemical properties of loess soil. *Journal of Soil Science and Plant Nutrition*, 15(3), 711-725.
- Zhang, L., Chen, L.J., Chen, X.H., Tan, M.L., Duan, Z.H., & Wu, Z. (2015b). Response of soil enzyme activity to long-term restoration of desertified land. *Catena*, 33, 64-70.

**Publisher's Note:** ASEA remains neutral with regard to jurisdictional claims in published maps and figures.