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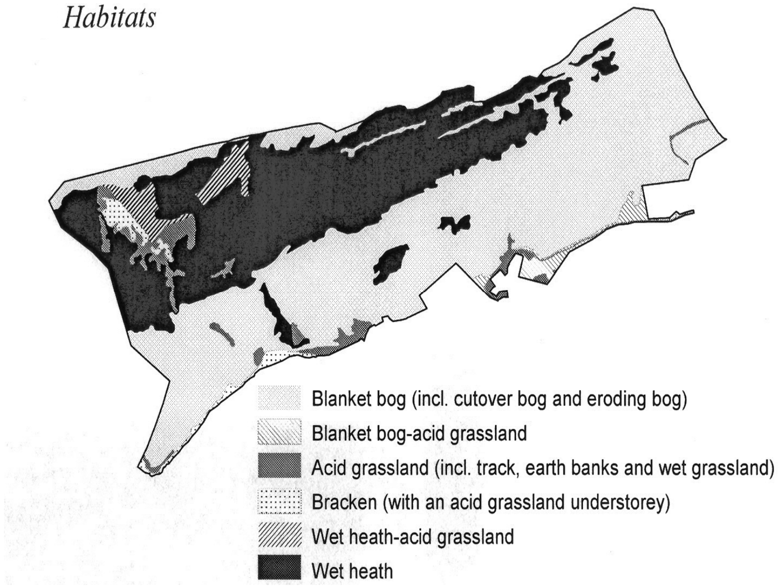

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RESOURCE SELECTION BY HILL SHEEP: DIRECT FLOCK OBSERVATIONS VERSUS GPS TRACKING

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Abstract. Uplands and peatlands are of international ecological importance and heavy grazing pressure has been implicated in a decline in their condition. Therefore, a better understanding of grazer behaviour could aid the design of conservation strategies. The objective of this study was to test whether the same resource selection results were obtained from direct flock observations as simultaneously-run GPS tracking studies. Direct flock observations were conducted on 50 sampling days, June 2004-July 2005. Habitat, habitat condition and grazing lawn frequency maps were produced. Resource use and availability were estimated using range analysis and GIS, and resource selection was analysed using weighted compositional analysis. Flock distribution was uneven with a mean of 0.0-8.9 sheep/ha observed based on a 1 ha grid system. Habitat selection based on direct observations varied seasonally with acid grassland-related habitats selected most in spring, summer and autumn, and wet heath and blanket bog selected most in winter. Moderately damaged areas and grid squares containing numerous/extensive grazing lawns were consistently selected most ($P<0.05$). Resource selection findings based on direct flock observations were consistent with those obtained using detailed GPS tracking data from fewer individuals. Resource management recommendations are discussed.

Keywords: *agriculture; ecology; environment; resource management; resource selection*

Introduction

Uplands and peatlands are of international importance for their associated flora and fauna and restricted global distribution and extent. Associated habitats, such as ‘temperate Atlantic wet heath with *Erica ciliaris* and *Erica tetralix*’ and active ‘blanket bog’, are recognised as priority habitats for conservation at a European level (European Commission, 2008). Heather-dominated landscapes are reported to be best represented in the British Isles (Thompson *et al.*, 1995b), which also supports 18-22% of the world’s blanket bog (Cadbury, 1987, Department of Arts Heritage Gaeltacht and the Islands, 2002). However, grazing-related damage to European uplands is most notable in the UK and Ireland (European Environmental Advisory Councils, 1999) where sheep farming is the prevalent land use. This damage is probably attributable to relatively high sheep numbers; of nearly 26 million breeding sheep in northern Europe, 80% are on the British Isles (Dýrmundsson, 2006).

EU policy has a large influence on the management of areas with poor soil and climatic conditions for agricultural production, such as upland areas. Irish sheep farmers (mainly hill sheep farmers, L. Connolly *pers. comm.*) are highly dependent on direct payments and additional subsidies, for example, excluding subsidies, production costs exceeded incomes generated in 2006 (Connolly *et al.*, 2007). EU policy in recent decades encouraged high stock numbers which resulted in grazing-related damage (Gillmor and Walsh, 1993), conversely, there is now increasing concern that changes in financial support will lead to undergrazing and land abandonment in European uplands (Moravec and Zemeckis, 2007). Grazing animals are widely recognised as an important management tool in ecological conservation (Rowell and Clarke, 1988, Thompson *et al.*, 1995a) and agri-environment schemes provide support to hill farmers that is essential if grazers are to be kept on upland areas. A better understanding of grazer behaviour could increase the efficacy of agri-environment schemes.

Provenza and Cincotta (1993) recognised the difficulty in predicting foraging behaviour. A complex computer-based hill grazing management model of sheep stocking regime impacts on hill vegetation has been developed (Armstrong *et al.*, 1997a; Armstrong *et al.*, 1997b). This model has been used by various organisations to aid decision-making (Hester and Baillie, 1998) and a new version of this model, which incorporates additional components, is awaiting further data for validation before release (HillPlan; Macaulay Institute, 2009).

Grazer preferences have been investigated using analysis of faecal, stomach and oesophageal samples (Heady, 1964; Williams, 1969; McInnis *et al.*, 1983). Seasonal diet selection of hill sheep has been reported in several studies (Welch, 1984; Bullock, 1985; Grant *et al.*; 1985, Grant *et al.*, 1987). Vibracorders, sensors built into GPS collars and video recordings have been used to investigate sheep behaviour including activity, circadian rhythm and inter-animal distances (Hulbert *et al.*, 1998; Iason *et al.*, 1999; Sibbald *et al.*, 2000; Umstätter *et al.*, 2008). Direct observations of hill sheep behaviour activity, location and orientation have also been made (Hunter, 1962; Berggren-Thomas and Hohenboken, 1986; Clarke *et al.*, 1995; Hester *et al.*, 1999).

Scottish Blackface is the dominant hill sheep breed in the British Isles and, therefore, the obvious choice as the study animal. Informative studies of habitat selection by hill sheep have previously been based on direct observation data at the very detailed plant community level (Hunter, 1962) or within 2.5 ha plots and with only two habitats available (Clarke *et al.*, 1995). The former has practical limitations for large-scale repeatability and the latter excludes a number of habitats typically available on heterogeneous hill areas.

Key findings from studies by the same authors, which used GPS tracking collars on hill sheep with access to a mosaic of upland and peatland habitats, are that sheep; (i) occupied only 9-20% of the area available to them, (ii) selected acid grassland most and avoided blanket bog, (iii) mostly selected 1 ha grid squares that contained numerous/extensive grazing lawns (patches of grassland with short, dense swards that have been heavily grazed (McNaughton, 1984) and (iv) did not avoid severely damaged areas with comparatively low forage availability (Williams, 2008; Williams *et al.*, 2009).

The objectives of this study are (i) to investigate resource selection based on direct flock observations and (ii) to compare these results (that have a relatively large sample size) with findings from GPS tracking collars on a limited number of sheep (Williams, 2008; Williams *et al.*, 2009) which were made at the same time. The implications for conservation strategies using the two methods are also discussed.

Methods

Study area

The study area consisted of 216.9 ha of upland and peatland at the Teagasc Hill Sheep Farm in Co. Mayo, Ireland (53°37'N, 09°41'W). The dominant habitats were blanket bog and wet heath, with fragmented patches of acid grassland. The study area was within the catchment area of the Erriff River and part of the Mweelrea/Sheeffry/Erriff Complex candidate Special Area for Conservation and proposed Natural Heritage Area. The general aspect was south-southeasterly and the altitude ranged from 15-275 m OD, with the highest, steepest slopes in the northwest corner of the study area. The site was Class 5 for agricultural land use (Gardiner and Radford, 1980). Soils were mainly peats and peat depths ranged between 30 and 525 cm (Walsh *et al.*, 2000).

A maritime temperate climate prevailed with the nearest synoptic meteorological station located in Belmullet, Co. Mayo, approximately 70 km distant. Based on the most recent 30-year averages (1961-1990) recorded at Belmullet, the mean daily temperature was 14.0 °C in July and 5.7 °C in January, and the annual mean daily duration of bright sunshine was 3.5 h (Met Éireann, *undated*). The mean annual rainfall recorded on-site (1993-2005) was 2086.4 mm (L. O'Malley *pers. comm.*). The minimum and maximum hours of daylight at the study area were calculated as 7 h 27 min and 17 h 4 min.

Scottish Blackface sheep grazed the study area at stocking rates of 0.4 ewes/ha in spring (March-May), 0.9 ewes/ha in summer (June-August) and autumn (September-November) and 0.8 ewes/ha in winter (December-February). These calculations were based on 2004-2005 averages, omitted lambs and included hoggets at a ratio of 3 hoggets:2 ewes. The study area was grazed for 348 days in 2004 and 351 days in 2005. Supplementary feed was not given in the study area. Ewes lambed in early April with a productivity of 1.0 lamb/ewe (based on mean data 2004-2006). Approximately 80 females were retained annually as replacements. (L. O'Malley *pers. comm.*). Sheep had open access across the site, which was marked with pegs in a 100 × 100 m (1 ha) grid.

Direct flock observations

Sheep locations and behaviour activities were recorded for individuals at the time of sighting. Locations were estimated to the nearest grid square and behaviour activity categories used followed those of (Hester et al., 1999), i.e. grazing, moving, standing, lying, interacting and other ('other' activities included defecating, scratching against a post and the shaking of rain water from fleeces).

Flock observations were made from along a set route, approximately 8.2 km in length, designed (i) to minimise disturbance to sheep before recording locations and behaviour activities and (ii) to gain sight over most of the study area. Flock observations began at 10:00 local time and took approximately 5 hours to complete. Recordings were made in all weather conditions using a Dictaphone sealed in a ziplock plastic bag and transcribed to an Excel spreadsheet. Flock observations were conducted twice weekly for five weeks for each of five seasonal sampling periods between June 2004 and July 2005, providing a total of 50 sampling days.

Habitat, habitat condition and grazing lawn frequency surveys

Habitats were mapped using the UK and Irish classifications and guidelines (Nature Conservancy Council, 1990; Fossitt, 2000; Heritage Council, 2002) to enable comparison with other studies, increased replicability and further applications. Habitat patches ≥0.25 ha were mapped with the aid of colour, ortho-corrected aerial photographs taken in 2000 (Ordnance Survey Ireland, Dublin, Ireland). Habitats are referred to throughout by the Irish classification names, the UK equivalents are detailed in Williams (2008).

Habitat condition of the study area was originally mapped in (Bleasdale and Heffron, 1999) and ground-truthed for change in 2005. Assessment was made following the method described by Dúchas the Heritage Service and the Department of Agriculture and Food (1999) which uses six condition categories; undamaged, moderate-undamaged, moderately damaged, moderately-severely damaged, severely damaged and very severely damaged. Habitat condition indicators include (but are not exclusive to) grazing-related damage, are defined for each habitat type, and include vegetation cover and growth, particularly the cover and condition of *Calluna vulgaris* (L.) Huds and the cover of *Nardus stricta* (L.), species richness, sward height, exposed soil and evidence of burning.

All acid grassland patches were grazing lawns, a product of intensive grazing, but acid grassland patches were under-represented on the habitat map because patches were fragmented and often too small to map following mapping guidelines. To efficiently obtain more information on acid grassland distribution, grazing lawn frequency by grid square was mapped. Grazing lawn frequencies were allocated for each full or part 100×100 m grid square in 2005. Categories of grazing lawn patches (~4-7 m^2 or the equivalent area) were (i) none, (ii) few (1-5 patches), (iii) several (6-10) and (iv) numerous/extensive (>10). In the instance of a part grid square, on the boundary of the study area, it was classified as numerous/extensive if acid grassland occupied ≥0.3 of its area.

The habitat, habitat condition and grazing lawn frequency maps were digitised using Geographical Information System (GIS) software (ArcGIS Desktop, V.9.1 & 8.3, ESRI, Redlands, CA, USA).

Data analysis

Resource analysis

The habitat, habitat condition and grazing lawn frequency maps were imported into range analysis software (Ranges7, Anatrack Ltd, Dorset, UK) and analyses performed to estimate resource content of the study area. Availability and flock use of these resources based on grid squares was estimated using ArcGIS (including the identity tool) and Excel (including pivot tables).

Statistical analysis

During the daily 5 hour direct flock observations, visibility conditions (high or low) were recorded. Spearman's rank correlation test (implemented using SPSS v.15, Chicago, Illinois, USA www.spss.com) was used to test whether there was a correlation between visibility conditions and the proportion of the flock sighted.

Selection of habitat, habitat condition and grazing lawn frequency categories was examined using weighted compositional analysis (Compos Analysis V.6.2+, Smith Ecology Ltd, Abergavenny, UK). Each sampling day was weighted by the number of observations. Proportions of resource use were compared with those available, using Wilks' lambda (Λ) test (MANOVA). (Aebischer et al., 1993) used a two-step approach based on Johnson's selection levels (Johnson, 1980), which was previously implemented with the GPS data, comparing (i) habitats available within the study area with those used within ewe ranges (broad level) and (ii) habitats available within ewe ranges compared with those used at location (detailed level), (Williams, 2008). This method was repeated with GPS data for habitat condition and grazing lawn frequency (Williams *et al.*, 2009). However, with the flock observation data, only a one-step approach could be used, comparing resources available within the study area with those used at the 1 ha grid square level. This estimate is intermediate between the broad and detailed levels because there is no precise location data and most individuals were unmarked prohibiting ewe range estimates.

Compositional analysis tests are based on percentage values, which overcame the potential problem arising from the inclusion of part and complete grid squares. Resource use and available percentage data will not have an exact multivariate normal distribution, and hence randomisation tests were used to evaluate the significance of Λ and t values (Aebischer *et al.*, 1993).

All sampling days were combined for annual tests, with summer resource use and number of observation values averaged because this season was duplicated. To enable comparison with results previously obtained from a limited number of sheep tracked using GPS simultaneous to these direct flock observations, tests were repeated using three habitat and three habitat condition groups (*Table 1a,c*). Where the two combination habitats (characteristics of two habitats were exhibited in these instances, i.e. blanket bog-acid grassland and wet heath-acid grassland) overlapped two habitat groups, values were divided equally between the two corresponding habitat groups.

A total of 8791 sheep observations were made over the 50 sampling days. All were suitable for analysis of sheep behaviour activities and proportions of the flock sighted. Sixty-five observations were omitted from compositional analyses as these were of sheep that chose to occupy a fenced exclosure outside the study area and were unsuitable for resource selection analysis because different management practices in the exclosure affected classification of habitats, habitat condition and grazing lawn frequency.

Table 1. *Availability of habitats, grid squares containing different frequencies of grazing lawns and areas of differing habitat condition in the 216.9 ha study area*

Category	Abbreviated name/code	Area (%)	Group
a) Habitat			
Blanket bog	Blanket bog	52.8	Bog
Wet heath	Wet heath	35.3	Heath
Semi-natural dry-humid acid grassland	Acid grassland	3.0	Grassland
Wet heath-Semi-natural dry-humid acid gsld	Heath-grassland	2.4	Heath & grassland
Cutover bog	Cutover bog	2.0	Bog
Dense bracken	Bracken	1.6	Grassland
Blanket bog-Semi-natural dry-humid acid gsld	Bog-grassland	1.6	Bog & grassland
Eroding blanket bog	Eroding bog	0.5	Bog
Semi-natural wet grassland	Wet grassland	0.5	Grassland
Buildings and artificial surfaces	Track	0.4	Grassland
Earth banks	Earth banks	0.1	Grassland
b) Grazing lawn frequency			
None	None	11.4	N/A
Few (0-5)	Few	36.8	N/A
Several (6-10 or equivalent)	Several	17.4	N/A
Numerous (≥10)/extensive	Numerous	34.3	N/A
c) Habitat condition			
Undamaged areas	U	29.5	Undamaged (U)
Moderate-undamaged areas	MU	22.3	Moderately damaged (M)
Moderately damaged areas	M	21.0	Moderately damaged (M)
Moderate-severely damaged areas	MS	5.5	Moderately damaged (M)
Severely damaged areas	S	2.0	Severely damaged (S)
Very severely damaged areas	VS	19.8	Severely damaged (S)

Results

Direct flock observations

A mean of 79.1 % (min. 57.6 %, max. 94.6 %) of the flock was sighted on 50 sampling days. The Spearman rank test indicated that the proportion of the flock sighted was not affected by visibility conditions (correlation coefficient=0.168, P=0.245, n=50).

Grazing was the main behaviour activity observed with means of 63.1-94.6 % of sightings per sampling period (*Fig. 1*). Lying was the second-most observed activity with means of 2.5-30.2 % of sightings per sampling period. Proportions of sheep sighted grazing were highest in winter. The highest proportions of sheep sighted lying were in both summers and autumn. The number of observations was low in spring because ewes were taken off the study area and given supplementary feed prelambing if twin-bearing or of low body condition.

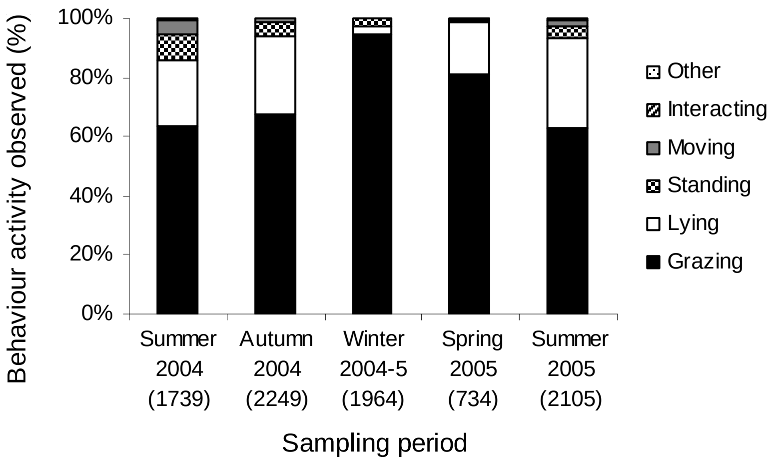


Figure 1. *Mean proportions of behaviour activities of Scottish Blackface hill sheep observed on 10 days for each of five seasonal sampling periods. The total number of sheep observations made in each sampling period is included in x-axis labels*

Flock distribution across the study area was clearly uneven (*Fig. 2*). Five categories, with four manual breaks in sheep density values, were used to identify (i) unoccupied grid squares (0.00), (ii) squares occupied at or below densities expected based on the annual stocking rate of 0.74-0.75 ewes/ha (L. O'Malley *pers. comm.*), (iii) squares occupied at densities greater than expected based on the annual stocking rate (>0.75, subdivided into two categories using the mean midpoint between 0.75 and the highest mean observed density). Sheep densities were lower overall in spring as most of the flock was brought in for supplementary feed prelambing. Sheep densities were higher overall in summer as sheep numbers observed included hoggets and lambs whereas annual stocking rate calculations excluded lambs and included hoggets as ewes at a 3:2 ratio (L. O'Malley *pers. comm.*). A preference for the northwest quarter of the study area was most marked in summer (2004 and 2005) compared with alternative seasons. Between season and year variation was evident in flock distribution, although distribution was most similar for the two consecutive summers sampled.

The mean sheep density by grid square across all 50 sampling days indicated a preference for the northwest quarter and northeast corner of the study area (*Fig. 3a*). Mean densities per sampling period and overall are presented in *Figures 2* and *3a* but absolute sheep densities by grid square for the 50 sampling days found observed densities ranged between 0.00 and 158.13 sheep/ha with a mean density of 0.93 sheep/ha. However, the highest observed sheep density for each sampling period was consistently found in part grid squares on the boundary of the study area ranging in size between 0.05 and 0.13 ha. The highest number of sheep seen in a grid square at any one time was 23 and this occurred in complete, 1 ha grid squares on two occasions (i.e. a density of 23.00 sheep/ha).

On any one sampling day, sheep were observed to be dispersed across the study area, as opposed to being congregated in one group. Frequency histograms indicated that typically the majority of grid squares were unoccupied, none were occupied at low densities between 0.01 and 0.93 sheep/ha, and sheep occupation was at densities of ≥0.94 sheep/ha (*Fig. 4*).

***Figure 2.** Mean sheep density by grid square from 10 days for each of five seasonal sampling period*

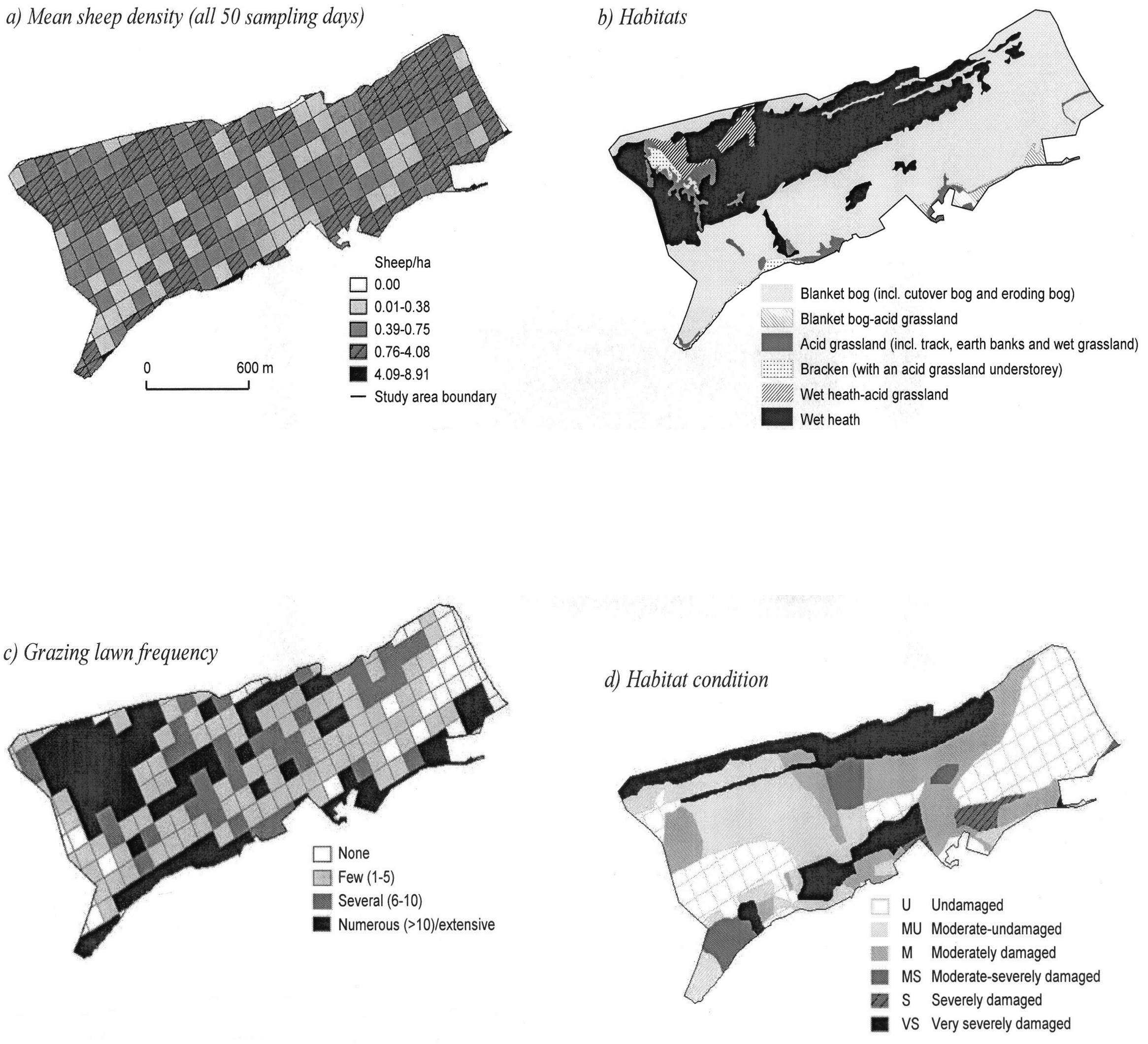
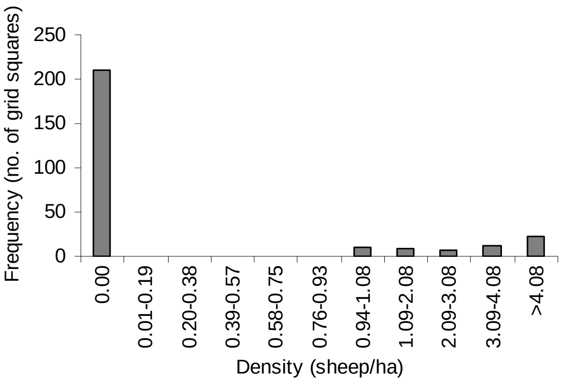


Figure 3. *Spatial distribution maps of sheep occupation, habitats, grazing lawn frequencies and habitat conditions for the study area.*

a) Sampling day no. 8 of 10, autumn 2004



b) Sampling day no. 4 of 10, summer 2005

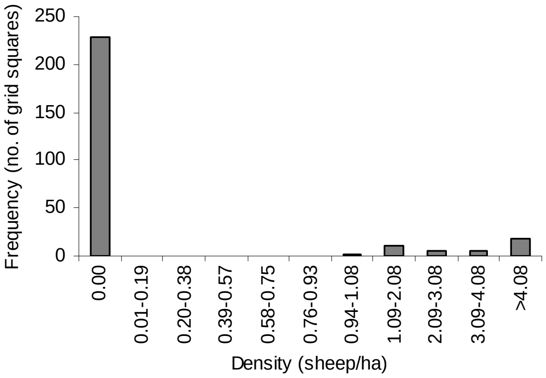


Figure 4. *Sheep densities observed in the study area which consisted of 270 grid squares. These are typical frequency histograms from two randomly-selected sampling days.*

Data presented in sub-sections are based solely on direct flock observations.

Habitat selection

Eleven polygon habitats were available to grazers (*Table 1a*). The dominant habitats were blanket bog and wet heath, which combined accounted for 88.1% of the study area (*Table 1a*, *Fig. 3b*). Wet heath was mostly associated with the slopes of the northern half of the study area and blanket bog with the more level areas, typically at lower altitudes.

Table 2. *Tests for random use of habitats by Scottish Blackface hill sheep based on direct observations. The habitat ranking is shown in parentheses when* Λ *is not significant and '>>>' denotes a significant difference between two consecutively ranked habitats.*

Sampling period(s)	Randomness test Λ	P	Habitat rankings (most>least selected)	No. of sheep sightings	% of total sightings
a) Tests with nine habitats					
Annual 2004-5	0.308	≤0.001***	Bracken>Acid grassland>Track>Bog-grassland>Wet heath>>>Blanket bog>Heath-grassland>Cutover bog>Wet grassland	8692	99.6
Summer 2004	0.101	0.324	(Bracken>Acid grassland>Heath-grassland>Track>Wet heath>Bog-grassland>Blanket bog>Cutover bog>Wet grassland)	1728	99.5
Autumn 2004	0.010	0.040 *	Acid grassland>Track>Heath-grassland>Bracken>Cutover bog>Bog-grassland>Wet heath>Blanket bog>Wet grassland	2223	99.9
Winter 2004-5	0.192	0.558	(Wet heath>>>Blanket bog>Track>Bracken>Acid grassland>Heath-grassland>Bog-grassland>Cutover bog>Wet grassland)	1954	99.8
Spring 2005	0.047	0.178	(Bracken>Track>Bog-grassland>Acid grassland>Wet heath>Blanket bog>Heath-grassland>Wet grassland>Cutover bog)	721	99.3
Summer 2005	0.023	0.073	(Bracken>Heath-grassland>Acid grassland>Bog-grassland>Track>Wet grassland>Cutover bog>Wet heath>Blanket bog)	2066	99.3
b) Tests with three habitat groups					
Annual 2004-5	0.519	≤0.001***	Grassland>>>Heath>>>Bog	8726	100.0
Summer 2004	0.251	0.019 *	Grassland>>>Heath>>>Bog	1737	100.0
Autumn 2004	0.520	0.086	(Grassland>>>Heath>Bog)	2226	100.0
Winter 2004-5	0.463	0.021 *	Grassland>Heath>>>Bog	1957	100.0
Spring 2005	0.200	0.003 **	Grassland>>>Heath>Bog	726	100.0
Summer 2005	0.109	0.002 **	Grassland>>>Heath>Bog	2080	100.0

Habitat selection tests (*Table 2a*) were conducted with only nine of the 11 polygon habitats because earth banks and eroding bog had to be omitted since too many zero values, indicative of low use, prohibited analyses running. Habitat selection by sheep was only significant ($P<0.05$) for autumn and annual tests, although habitat ranks were still considered to be meaningful (Williams, 2008). Bracken (*Pteridium aquilinum* L. Kuhn) was selected most in both summers and spring, and acid grassland was selected most in autumn. Wet heath followed by blanket bog were most selected in winter whereas these two habitats were of intermediate to low rank in the other seasons. No two rank sequences were identical, indicating variation between seasons and years.

To include all polygon habitat data and to simplify comparison with results previously obtained from GPS tracking of ewes (section 3.5), the 11 habitats were combined into three groups (*Table 1a*) and tests re-run. Habitat group selection was significant ($P<0.05$) for all tests except autumn (*Table 2b*). The selection rank for all tests was consistently acid grassland-related habitats selected most, followed by wet heath, and blanket bog habitats selected least.

Grazing lawn frequency selection

Grid squares containing few (36.8 %) and numerous/extensive (34.3 %) grazing lawns were the most frequently occurring (*Table 1b*). The largest block of grid squares containing numerous/extensive grazing lawns was found in the northwest corner of the study area, some were also located along the southern boundary and scattered elsewhere (*Fig. 3c*).

Selection of grid squares containing different frequencies of grazing lawns was significant ($P<0.05$) annually and for all seasons except winter (*Table 3*). All four categories were suitable for analysis and did not lend themselves to further grouping as with habitat selection analyses. Grid squares containing numerous/extensive grazing lawns were selected most overall and in all seasons tested. The rank of the subsequent three categories varied slightly between seasons and years.

Table 3. *Tests for random use, by Scottish Blackface hill sheep, of grid squares consisting of varying grazing lawn frequencies (based on direct observations). The grazing lawn frequency ranking is shown in parentheses when Λ is not significant and '>>>' denotes a significant difference between two consecutively ranked frequency categories.*

Sampling	**Randomness test**		**Grazing lawn frequency rankings**	**No. of**	**% of total**
period(s)	**Λ**	***P***	**(most>least selected)**	**sightings**	**sightings**
Annual 2004-5	0.459	≤0.001***	Numerous>>>Several>None>Few	8726	100.0
Summer 2004	0.135	0.008 **	Numerous>None>Several>Few	1737	100.0
Autumn 2004	0.304	0.035 *	Numerous>None>Several>Few	2226	100.0
Winter 2004-5	0.908	0.877	(Numerous>None>Several>Few)	1957	100.0
Spring 2005	0.144	0.013 *	Numerous>>>Several>>>Few>None	726	100.0
Summer 2005	0.069	0.002 **	Numerous>>>Several>None>Few	2080	100.0

Habitat condition selection

All condition categories from undamaged to very severely damaged areas occurred in the study area (*Table 1c*). The most dominant single category available was undamaged areas (29.5 %). Moderately damaged categories combined accounted for 48.8 % of the study area and severely damaged categories combined accounted for 21.8 %. A mosaic of habitat conditions was found (*Fig. 3d*). Severely damaged areas were most associated with the mountain ridge and, in places, at low altitudes. Moderate damage was mostly associated with the northern half of the study area and undamaged areas were mostly associated with more level areas at low altitude.

Selection of areas with varying habitat condition, using all six categories, was significant ($P<0.05$) annually and for all seasons except winter (*Table 4a*). Moderate-undamaged areas were selected most in both summers, autumn, spring and annually but second-least in winter when moderately damaged areas were selected most. Severely damaged areas were selected least in all tests except second-least in summer 2005. Very severely damaged areas had a low selection rank of five out of six in autumn, rose to four in spring, and were selected more in summer of both years and spring with a selection rank of three.

Table 4. *Tests for random use of habitat condition categories by Scottish Blackface hill sheep based on direct observations. The habitat condition ranking is shown in parentheses when Λ is not significant and '>>>' denotes a significant difference between two consecutively ranked condition categories.*

Sampling	**Randomness test**		**Habitat condition rankings**	**No. of**	**% of total**
period(s)	**Λ**	***P***	**(most>least selected)**[a]	**sightings**	**sightings**
a) Tests with six condition categories					
Annual 2004-5	0.446	≤0.001***	MU>M>U>>>VS>MS>>>S	8726	100.0
Summer 2004	0.169	0.036 *	MU>>>M>VS>U>MS>S	1737	100.0
Autumn 2004	0.036	≤0.001***	MU>U>M>MS>VS>S	2226	100.0
Winter 2004-5	0.154	0.053	(M>U>VS>MS>MU>S)	1957	100.0
Spring 2005	0.085	0.011 *	MU>>>M>U>VS>MS>S	726	100.0
Summer 2005	0.134	0.039 *	MU>M>VS>U>S>MS	2080	100.0
b) Tests with three condition groups					
Annual 2004-5	0.341	≤0.001***	M>>>U>>>S	8726	100.0
Summer 2004	0.710	0.223	(M>S>U)	1737	100.0
Autumn 2004	0.047	0.003 **	M>U>>>S	2226	100.0
Winter 2004-5	0.365	0.017 *	M>U>S	1957	100.0
Spring 2005	0.218	0.006 **	M>>>U>>>S	726	100.0
Summer 2005	0.444	0.040 *	M>>>S>U	2080	100.0

[a] U = undamaged, MU = moderate-undamaged, M = moderately damaged, MS = moderate-severely damaged, S = severely damaged, VS = very severely damaged areas.

The six condition categories were grouped into the three broader categories (*Table 1c*) defined by Dúchas the Heritage Service and the Department of Agriculture and Food (1999) and selection tests were re-run. Selection was significant ($P<0.05$) annually and for all sampling periods except summer 2004 (*Table 4b*). Moderately damaged areas were consistently selected most in all tests. Severely damaged areas were selected least annually and in autumn, winter and spring, whereas severely damaged areas were second-most selected in both summers.

While visual comparison of the four maps presented in *Figure 3* suggests there is not a clear, direct link between flock distribution, habitats, grazing lawn frequencies and/or habitat condition, in summary, compositional analysis results suggest that flock distribution was generally associated with acid grassland, numerous/extensive grazing lawns and moderately damaged areas.

Direct flock observations and GPS tracking results compared

Habitat rank sequences with three habitat groups based on direct flock observations matched those based on GPS data for all seasons at the detailed selection level and for summer and autumn at the broad level. When comparing flock observations with GPS data at the broad level with five habitats common to both sets of tests, there were similarities and differences. Examples of similarities include wet heath being selected most in winter and acid grassland selected most in autumn with blanket bog least, preceded by wet heath in both sets of results. Examples of inconsistencies in results between methods include blanket bog selected second-most in winter with flock observations but least with GPS data, and bracken selected most in spring with flock observations but second-least with GPS data.

Grid squares containing numerous/extensive grazing lawns were most selected in all tests, i.e. in all seasons and at all selection levels, based on direct flock observations and GPS tracking data (Williams *et al.*, 2009).

Moderately damaged areas were consistently selected most in tests using flock observation data and GPS data (Williams *et al.*, 2009). Both methods indicated a higher selection of severely damaged areas in summer (results from GPS data presented in (Williams *et al.*, 2009), although this was also found to be true in spring using flock observation data.

Discussion

Direct flock observations

The majority of the flock was sighted when following the chosen route. Complex topography with hummocks, hollows and steep slopes obscured some sheep from view and hindered observer access to some areas. Visibility did not affect proportions of the flock sighted.

Grazing was the main behaviour activity observed and more so in winter than other seasons, which is consistent with findings by others. Scottish Blackface sheep are reported to graze hill vegetation 79% of daylight time in winter (Hulbert *et al.*, 1998), 53% in summer (Clarke *et al.*, 1995) and 60% in summer and autumn (Hester *et al.*, 1999). Proportions of sightings are probably indicative of time spent on respective behaviour activities and values for summer and autumn are similar to those of (Hester et al., 1999). Feeding blocks were available in the trial by (Hulbert et al., 1998) which may explain the lower proportion of time spent grazing in winter compared with observations in this study where no supplementary feed was available.

Uneven sheep distribution and a preference for the northwest quarter of the study area are consistent with findings from GPS data (Williams, 2008) and are likely to be explained by resource selection as discussed below. Hill sheep, including Scottish Blackface, are thought to learn home ranges from their dams and are known to return to the same areas (Hunter and Milner, 1963; Lawrence and Wood-Gush, 1988). Foraging behaviour is also reported to be influenced by experiences of individuals (Provenza and Cincotta, 1993). Therefore, a feedback loop probably occurs between experience gained of environmental conditions, including habitats, grazing lawns and habitat condition, and home ranges subsequently passed on to offspring/learned. High occupation of the northwest quarter of the study area is consistent with findings that hill sheep prefer the area with the highest elevation (Berggren-Thomas and Hohenboken, 1986).

A striking finding is that sheep densities ranged between 0.00 and 158.13 sheep/ha (0.00-8.91 mean over 50 sampling days). Many studies of hill sheep behaviour are plot-based trials which provide valuable information but cannot explore sheep densities applicable to open hill situations. Conversely, Lawrence and Wood-Gush (1988) studied sheep behaviour on an open hill area but did not report on sheep densities. Hewson and Wilson (1979) reported a maximum of 35 sheep and 14 lambs on a heavily grazed coastal grassland area about 1.6 ha in size (equivalent to 30.6 sheep/ha). A comparison between Hewson and Wilson's study and the present study would be unfair because different methods were used, including the use of a 1 ha grid and more advanced technology (i.e. GIS software) in this study. Occasional highs of 16 sheep in 0.1 ha (equivalent to 158.1 sheep/ha but strongly influenced by grid positioning) and 23 sheep in complete, 1 ha grid squares (i.e. equivalent to 23.0 sheep/ha) were observed in this study. This information highlights the level of selection that can be exhibited by hill sheep on heterogeneous upland topography and associated vegetation.

The highest observed sheep density (158.13 sheep/ha) occurred in mid-October 2004 when the weather was dry, windy and overcast with high visibility. Sixteen sheep were observed occupying a grid square which was on the mid-northern boundary and only 0.10 ha in size, estimated using ArcGIS. The grid square was on the mountain ridge, with steep slopes and rock outcrops indicative of shallower soils than elsewhere, and dominated by wet heath.

Habitat selection

In tests with nine habitats, bracken was most selected in both summers and spring, probably because it supported an understorey of acid grassland, and acid grassland was selected most in autumn. Acid grassland-related habitats were consistently selected most in tests with three habitat groups. Preference for grassland on heterogeneous hill vegetation is consistent with findings by others (Welch, 1984; Clarke *et al.*, 1995; Hester *et al.*, 1999). Probable explanations for a preference of acid grassland include high vegetation productivity potential (McNaughton, 1984), higher protein and energy concentration and plant biomass (Doyle, 1979, Doyle, 1982) and higher digestibility and herbage intake on acid grassland than heath or blanket bog (Hodgson *et al.*, 1991).

Seasonal variation in habitat rankings based on nine habitats is probably explained by seasonal variation reported in plant palatability and hill sheep diets (Bullock, 1985, Grant *et al.*, 1987). For example, acid grassland patches in the study area are dominated by *Molinia caerulea* (L.) Moench (O'Dowd, 2005) which dies back in early autumn and this provides a likely explanation for the top selection rank of wet heath as opposed to acid grassland in winter. When habitats were grouped, the broad selection trends were found but detail at habitat level was lost which is probably why heath was second to grassland-related habitats in winter for this test. This highlights the importance of conducting analyses with separate habitats (and ensuring sample sizes are large enough to do so) in addition to habitat groups because wet heath and blanket bog habitats were most selected in winter, which has important management implications.

Blanket bog-related habitats were consistently selected least, or most ‘avoided’, in tests based on the three habitat groups. Likely explanations include low vegetation productivity, digestibility and herbage intake, and a deficiency in minerals (Hodgson *et al.*, 1991), combined with taller vegetation which sheep find more difficult to graze (Grant *et al.*, 1985). Additionally, features associated with this habitat that made it difficult for sheep to move through, included bog pools, surface water, drainage ditches and areas of quaking peat.

Minor variation in habitat selection rank sequences between tests based on direct flock observations and GPS tracking data may be explained by flock observation analyses being intermediate between broad and detailed selection levels tested with GPS data. However, trends were generally consistent and led to the same conclusions.

Grazing lawn frequency selection

Grid squares containing numerous/extensive grazing lawns were associated with a large bowl-like feature and adjacent hillock in the northwest corner, the riverbank on the western half of the southern boundary, farm tracks and earth banks along the eastern half of the southern boundary, and scattered hillocks, rock outcrops and/or sloping ground with well-drained soils. Absence of grazing lawns was typically associated with areas of waterlogged, deep, quaking peat on relatively level ground, and occasionally with very steep north-facing slopes inaccessible to sheep on the northern boundary. The distribution of grazing lawns is probably explained by sheep being reported to congregate and produce grazing lawns where vegetation productivity potential is high and to disperse from areas where potential is low (McNaughton, 1984).

Sheep most selected grid squares containing numerous/extensive grazing lawns in all seasons, which is consistent with findings based on GPS data (Williams *et al.*, 2009). A close association between flock and grazing lawn distribution was expected as grazing lawns are a product of intensive grazing. During the plant growing season, selection is probably explained by the relatively better forage quality and availability of acid grassland as discussed above. Selection for grid squares containing numerous/extensive grazing lawns outside the vegetation growing season, when forage quality of acid grassland is relatively lower, is probably explained by sheep known to return to learned home ranges and to concentrate grazing activity in close proximity to grassland patches rather than further away (Clarke *et al.*, 1995; Hester and Baillie, 1998). Additionally, grid squares typically supported and were often dominated by alternative habitats such as wet heath.

Habitat condition selection

Severely damaged areas were most associated with the mountain ridge, which is probably largely explained by wind-driven rain and its effect on soil erosion reported to be most severe close to the top of a hill (Choi, 2002). Severely damaged areas on the southern boundary at low altitude were associated with deep peat, relatively level ground, waterlogging most of the year, former peat extraction in places and poaching on routes used when sheep were gathered and released from the yard. Moderately damaged areas were mostly associated with steeper slopes dominated by wet heath where sheep paths traversed the hill. Undamaged areas were species poor, dominated by tall, rank purple moor-grass and were arguably undergrazed.

Grazers can facilitate damage to upland habitats, conversely, they increase diversity in plant species composition, height and structure and the fauna this supports (Rowell and Clarke, 1988). While increasing diversity has benefits, creation of acid grassland patches is at the expense of wet heath (van Eck *et al.*, 1984; Gillmor and Walsh, 1993; Thompson *et al.*, 1995b) which, unlike acid grassland, is listed under Annex I of the Habitats Directive. Some acid grassland patches are inevitable where livestock graze hill vegetation and the spread of grassland patches is likely (Clarke *et al.*, 1995).

Moderately damaged areas were selected most, which was consistent with GPS data. Relatively better forage quality associated with these areas, compared with severely damaged areas that support higher proportions of exposed soil and undamaged areas that support tall vegetation that is difficult to graze, provides a probable explanation.

Higher selection of severely damaged areas in summer is consistent with findings from GPS data (Williams *et al.*, 2009), although this study also found this to be true in spring. Use of severely damaged areas particularly during the vegetation growing season raises concerns as grazing-related pressure is likely to inhibit recovery in areas where the proportion of exposed peat is highest. Seasonal selective grazing for preferred species probably explains use of these areas as Scottish Blackface sheep are thought to be more selective in summer when environmental heterogeneity is highest (Lawrence and Wood-Gush, 1988). *Eriophorum spp.* (L.) and *Narthecium ossifragum* (L.) Huds) were abundant species on areas of exposed peat and (Grant et al., 1987) reported high proportions of these in the diets of Scottish Blackface sheep grazing blanket bog during the plant growing season.

Implications for conservation

An important finding from this study based on direct flock observations was that habitat, grazing lawn frequency and habitat condition selection trends matched those previously obtained from thousands of GPS tracking data for only a small number of individuals. This adds credence to findings from these two, very different research methods, indicating that both methods produced results that are likely to be representative of the flock. Additionally, it reinforces management recommendations previously made (including by Williams (2008) and Williams *et al.* (2009)). These recommendations were that; (i) stocking rate calculations should be based on grazer behaviour, relative proportions of habitats available and habitat condition, (ii) areas of habitats selected least (i.e. avoided; blanket bog habitats in this study) should be omitted from stocking rate calculations, (iii) additional measures, such as fencing, are probably also necessary to meet conservation objectives, (iv) stocking rates should not increase the number or extent of acid grassland patches at the expense of heathland, (v) grazing lawn frequency mapping could be used as an indicator of grazer distribution and subsequent grazer impact predictions, and (vi) additional information on grazer selection should be gathered and incorporated into hill management models such as HillPlan (Macaulay Institute, 2009) and used to aid decision-making.

Flock distribution ranged between grid squares that were unvisited and where very high stocking densities were observed on occasion. Uneven grazing pressure combined with all six habitat conditions occurring in the one study area, which was managed as a single unit, makes management planning problematic. Areas with different condition categories and carrying capacities require different management prescriptions but compartmentalising an upland/open hill site, e.g. using fencing, is labour intensive and expensive, neither of which are conducive to the hill sheep enterprise with high dependence on off-farm employment and low profit margins (Connolly *et al.*, 2007). Exclusion of grazers from targeted areas and sheep numbers calculated based on habitat availability, grazer selection and habitat condition, should facilitate vegetation recovery on exposed peat and reduce grazer impact on damaged areas. In this and similar instances, grazers should be excluded from extensive severely damaged areas, e.g. the area associated with the mountain ridge, and effects monitored. Gathering sheep is an essential practice for production and animal welfare and routes that cross blanket bog were unavoidable, therefore use of erosion control mats or other ground reinforcement technology to withstand and contain trampling pressure on localised eroded patches could be considered.

Seasonal selection rankings of habitats, grazing lawns and habitat conditions provide useful information that could be used to predict foraging behaviour and consequently grazer impact on vegetation. Seasonal grazing regimes should be devised that meet habitat conservation objectives at site-level based on grazer behaviour and habitat condition. Traditionally, hill sheep overwintered in paddocks on the lower hills in Wales (Cunningham, 1979). Habitats with higher conservation importance and lower carrying capacities were most selected in winter, therefore, it is recommended that grazers are removed from areas of semi-natural hill vegetation during this period. On sites where fields of improved agricultural grassland are unavailable, creation of such areas could be considered to provide conservation support areas to the majority of the upland and peatland contained. Typically hill sites in western Ireland that do not already have fields of improved grassland are unlikely to have suitable sites for either improved grassland or livestock housing, in which case selling sheep at the start of winter and buying replacements in spring may be the only feasible option if conservation objectives are to be met.

Recommendations made above should not compromise the financial viability of hill farming, i.e. if extra costs are involved, farmers should be compensated accordingly. This enterprise is highly dependent on agri-environment payments which should continue to pay farmers to manage land for conservation but with revised management agreements, increasing the efficacy of agri-environment schemes in this sector based on new findings on grazer behaviour. Further studies are required on multiple study sites, with varying topography and vegetation assemblages, to obtain sufficient data on resource selection by hill sheep for inclusion as parameters in management models. Although this study investigated hill sheep selection on a mosaic of upland and peatland habitats, the same applications could be made to aid conservation decision-making on a wide variety of assemblages of habitats, plant communities and vegetation condition, and with a range of study animals.

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LIGHT TRAPPING AS A DEPENDENT OF MOONLIGHT AND CLOUDS

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Abstract. We examined in our study the theoretical catching distance and the catching results of the Járfás and Jermy type light-traps in the function of the moon phases and the clouds. The clouds determine the theoretical catching distances of both the Járfás and Jermy type light-traps fundamentally. The ratio of theoretical catching distances of completely overcast and clear sky is approximately 2.4:1. This difference does not appear however in the catching results. The catching of Turnip Moth (*Agrotis segetum* Den. et Schiff.) in moonless hours is the most successful when the sky is totally through, if it is not raining. In opposition to this, the catch decreases by the increase of the cloud cover in moonlit hours. The most moths were found in the light-trap when the sky was almost clear. The increase of cloud cover results in a reduction of the catch. The number of the Macrolepidoptera individuals and species are higher when the sky is clear than overcast in the event both the all and low clouds. It was also found that the height of cloud base also modify the light-trap catch. If the cloud base is low the light-trap catch is also low, in the opposite it is high.

Keywords: *Light-trap, moon phases, clouds, catching distance*

Introduction

Most of the authors experienced a drop in the efficiency of trapping as a result of moonlight. According to (Williams, 1936) the reasons for a smaller catch at a Full Moon might be as follows: Moonlight reduces the activity of insects and so the active population accessible for the light trap is smaller, or the light of the lamp collects moths from a smaller area in moonlit environment.

The researchers' opinion differs, some of them ascribe the smaller catching distance, others ascribe the moderate trapping result which can be experienced at the time of the Full Moon to the reduced activity. Some researchers take the role of the clouds into consideration. The cloud cover moderates the obstructive effect of the moonlight according to more researchers.

The clouds and the moonlight joint effect were already studied by (Williams, 1936). To examine the influence of cloud (Williams, 1936) divide the nights in the lunar months in two ways.

- In accordance with the lunar month: nights during the week of Full Moon, nights during the weeks intermediate moon (first- and Last Quarters) and nights during the week of New Moon.
- In accordance with the clouds: as regards the state of the sky, into three divisions: nights with more than 90 % of the sky clear (=clear), nights with 10 -

90 % of the sky clear (= intermediate) and nights with more than 90 % cloudy (= cloudy).

The interrelation of these two main divisions gives nine possible combinations as follows:

- Full Moon – clear, Full Moon – intermediate, Full Moon – cloudy
- First- and Last Quarters – clear, First- and Last Quarters – intermediate, First- and Last Quarters – cloudy
- New Moon – clear, New Moon – intermediate, New Moon – cloudy.

The nights with heavy wind where eliminated from calculations.

According to (Williams, 1936) results there was a tendency for the clear to be lower than the cloudy and for the Full Moon to be lower than the New Moon. The division Full Moon – clear sky give in each case the greatest negative deviation, and the division New Moon – cloudy sky give the greatest positive deviation.

The ratio of catches in the Noctuidae is shown to be 2.7:1 when New Moon was compared to Full Moon and 11.75:1 when cloudy sky are compared with clear sky. The ratio between New Moon – cloudy and Full Moon – clear is 4:1. Finally the relation New Moon – clear sky gives larger catches than Full Moon – cloudy sky 1.35:1.

Robertson (1939) reports on similar results. The cloud and moonlight have a marked effect on the light trap catches of Tipulinae. Optimum conditions are ensured by the absence of moonlight and a complete cloud cover. By contrast the conditions of Full Moon and the absence of clouds are the least favourable for the activity. Between these extremes, there is a consistent increase in catch with the increasing absence of moon and presence of clouds. The catch under optimum conditions is eight or nine times as large as in the least favourable conditions.

Garcia (1978) worked with light-traps in Venezuela, between 1973 and 1974. The largest catches of Sphingids were during the period when the moon was waning and the least catch was during Full Moon period. The cloud cover increased the effectiveness of the catch.

Bowden (1982) described the collecting radius of three different lamps with the same illumination. He also tabulated the correction values for the codes of the 10 categories of cloud types, according to which the catch rises with the increase of cloud cover.

In southern Spain, Yela and Holyoak (1997) investigated the effects of moon-phase on activity of adult Noctuid moths using light trap for 170 nights over of 2 year period. The number of individuals caught in the light trap decreased during the Full Moon period. Increased cloud cover increased catches in light traps. Cloud cover decreases the ambient light from the moon, making the light traps more visible.

According to Butler et al. (1999), the moonlight in the absence of cloud cover reduced moth catch in blacklight traps.

The most successful light trap collections were experienced by Robert (2001) during New Moon phase or on overcast nights. Mosquitoes came to lights in greatest numbers when nights were cloudy and smaller numbers when nights were clear and the moon was bright.

According to McCormick (2006-2007) the Bogong Moths (*Agrotis infusa* Boisduval) migrate and fly actively under cloudy conditions and during a New Moon.

Other researchers however experienced that clouds increases the catching distance though, but does not increase the efficiency of light trapping, but indeed reduces it.

Wéber (1959) depicted graphically the measure of a cloud cover and the number of insects caught. Unambiguously he established a contrary relationship, in other words the more the clouds covered the sky the fewer insects assembled to the light.

Edwards (1961) operated a Robinson-type UV trap during 93 nights to collect Huhu Beetle (*Prinoplus reticularis* Whitem, Col.:Ceramb.) in New Zealand. He describes an example in which the activity of beetles increased temporarily during Moon rise, followed by a decrease when the moon was obscured by clouds.

Járfás (1969) found that the silver Y moth (*Autographa gamma* L.) flight to light-trap better when there was no cloudy the sky. He observed a similar light flying on moonlit nights as well. Járfás and his colleague (Járfás and Viola, 1978, 1982) published similar statements in their later works onto the Dark Fruit-tree Tortrix (*Pandemis heparana* Den et Schiff.), and Pine Chafer (*Polyphylla fullo* L.) relevantly. Járfás (1979) experienced growing light-trap catch of codling moth (*Cydia pomonella* L.) when the measure of the cloud cover decreased. The light-trap caught most moths (57%) at clear sky. The higher catching results in moonlit and clear nights are linked to the orientation of the insects presumably. Flying of moths may increase to the light-trap together with the dusk while there are free areas of a cloud cover on the sky, because to the light compass orientation the insect receives enough light. If the light compass orientation of insects becomes impossible, the insect switches to orientation an other manner. This is not the inside truck for flying onto the light. The light-trap caught about three times of European Corn Borer (*Ostrinia nubilalis* Hbn.) at clear sky than full cloud cover.

Cordillot (1989) reports that clouds influences the light trapping of the European Corn Borer (*Ostrinia nubilalis* Hbn.) unfavourably because presumably it hampers their visual orientation.

The studies discussed above show that despite several decades of research into the influence of the moonlight and clouds on light trap catch, our knowledge in this field remains insufficient.

Materials

The necessary data required to our work were calculated with our own software. This software for our earlier research (Nowinszky and Tóth, 1987) was carried out by the late astronomer György Tóth for TI 59. The software was transcribed for modern computers by assistant professor Miklós Kiss: for which we express our sincere appreciation. The software calculates the phase angle of the Moon and its position above the horizon, the illumination (lux) taking the cloud cover into consideration for any given geographical place, day and time.

All our data regarding to all and low clouds and height of cloud base related data were taken from the Annales of the Hungarian Meteorological Service. Data in these books are recorded for every 3rd hour in okta (eighth part). We have used the value given for a given hour as well as for the subsequent two hours.

We have also processed catch data of the fractionating light trap of Kecskemét on 5722 individuals of the Turnip Moth (*Agrotis segetum* Den. et Schiff.). This special light trap system was designed and operated by József Járfás between 1967 and 1969 at Kecskemét-Katonatelep. The light source of the fractionating light trap consists of 3 pieces of 120 cm long F-33 type 40W light tubes placed above each other. Independent from the time of dusk and dawn, the trap was operated every day between 6 p.m and 04 a.m (UT). Killing jars were changed hourly by a jar-switching mechanism. These trapped insects were identified by József Járfás.

For our research, we used the complete Macrolepidoptera material of the Jermy type light trap (Jermy, 1961) operated in the Kámon Botanical Garden in Szombathely between 1962 and 1970, including data of 3395 adult broods. The light source is a 100W normal electric bulb 200 cm above the ground. Chloroform was used for the killing. This light-trap caught 37711 moths on 1980 nights.

Methods

First we determined the theoretical collecting distances of the Járfás- and Jermy type light-traps for the New Moon and Full Moon periods. For this test, the 5th August 1967 (New Moon) and the 19th August 1967 (Full Moon), dates were taken into account. We calculated the values of environmental illumination (lux) on 23 hours of both nights into attention the cloud codes. From these environmental illumination values we defined the theoretical catching distances. We compared our actual catching results with the theoretical catching distances.

The Jermy type light-trap provided one catching data for one night only. The phase angle data of the Moon was calculated for every midnight of the flight periods (UT = 0 h), and – in the case of fractionating light traps – for the 30th minute of every hour. Of the 360 phase angle degrees of the full lunar 30 phase angle divisions were established. The phase angle division including a Full Moon (0º or 360º) and values 0 ± 6º was equated to 0. Beginning from this group through the First Quarter until a New Moon, divisions were marked as -1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13 and -14. The next division is ±15, including the New Moon. From the Full Moon through the Last Quarter towards the New Moon divisions, were marked as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14. Each division consists of 12 degrees (Nowinszky, 2003). These phase angle divisions can be related to the four quarters of lunation as follows: Full Moon (-2 – +2), Last Quarter (3 – 9), New Moon (10 – -10) and First Quarter (-9 – -3). The nights and hours of the periods under examination were all classified into these phase angle divisions. We have also separated the hourly catching nights of fractionating light traps with or without moonlight.

The Jermy type light-trap supplied only one catching data nightly. Because of this in this case we calculated the data of phase angle onto the midnight (UT = 0 hour) of every night of the swarming periods.

We have calculated the relative catch values of the number of specimens trapped by species, broods and hourly data also separately for hours with and without moonlight from the fractionating light-trap data. Relative catch (RC) is the ratio of the number of specimen caught in a given sample unit of time (1 hour or 1 night) and the average number of specimen caught in the same time unit calculated for the whole brood. If the number of the specimen trapped equals the average, the value of relative catch is: 1.Only those nights and hours were taken into consideration when the catch was successful. Our earlier research (Nowinszky, 2003), convinced us that although the Moon has an influence on the efficiency of trapping, it never makes collecting impossible.

We included the relative catch values to the codes of catching hours averaged, and then separately for the moonlit and the moonless hours, then they were represented. We have given the parameters of regression equations and significance levels.

From the Jermy type light-trap data the nightly averages of the individuals and species catch were calculated.

We examined first around the Full Moon, First- and Last Quarter the influence of cloud for the catching. We sorted the okta codes in three groups according to the measure of the cloud cover. These are the following: clear (0, 1, 2), intermediate (3, 4, 5) and cloudy (6, 7, 8). Number of individuals and species caught were sorting in okta groups, were averaged and the significance levels of differences are counted with t-test.

To the additional examinations we selected the period of the Full Moon (phase angle divisions -2, -1, 0, +1 and +2) from the catch data because the Moon in 85.3 % are at this time stayed above the horizon. We do not consider the catching data of the periods of New Moon when there is not measurable moonlight and the data of the First- and Last Quarters, too. Not known the insects trapping in which hour of the night happened, and not know it that the Moon stayed above the horizon then, or not. We excluded the nights on which was rain, independent of his quantity from the examination. We sorted the nightly catching average values of the individuals and species to all the clouds, okta codes belong to the 2000 metres lower clouds and the values of height of cloud base (metres). We depicted the results on graphs, granted the regression equations parameters and the significance levels.

We included the relative catch values of both examined species to the hours of the catching owing okta codes, we averaged them, then separate hours without the moonlit one and the moonlight, we depicted them.

Results

The *Fig.1* and *Fig. 2* shows the theoretical catching distances of Járfás- and Jermy type light-traps in the surrounding of New Moon and Full Moon, depending on the clouds.

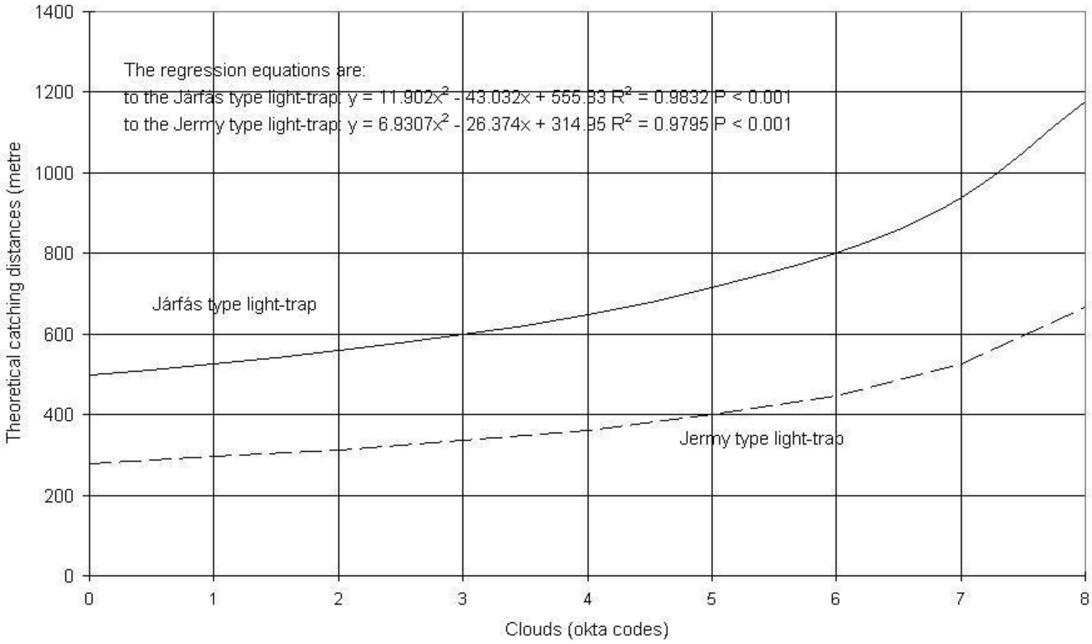

Figure 1. *Theoretical catching distances of the Járfás and Jermy type light-traps depending on the clouds around the New Moon*

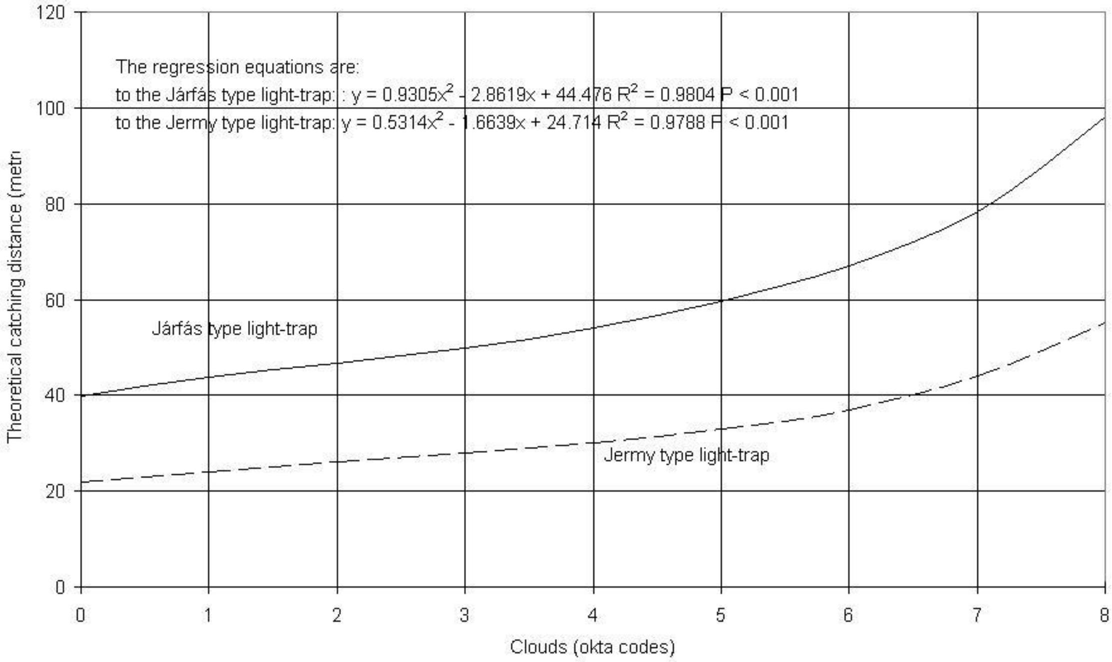

Figure 2. *Theoretical catching distances of the Járfás and Jermy type light-traps depending on the clouds around the Full Moon*

The catching results of the Turnip Moth (*Agrotis segetum* Den. et Schiff.) can be seen in *Fig. 3* and *Fig. 4*

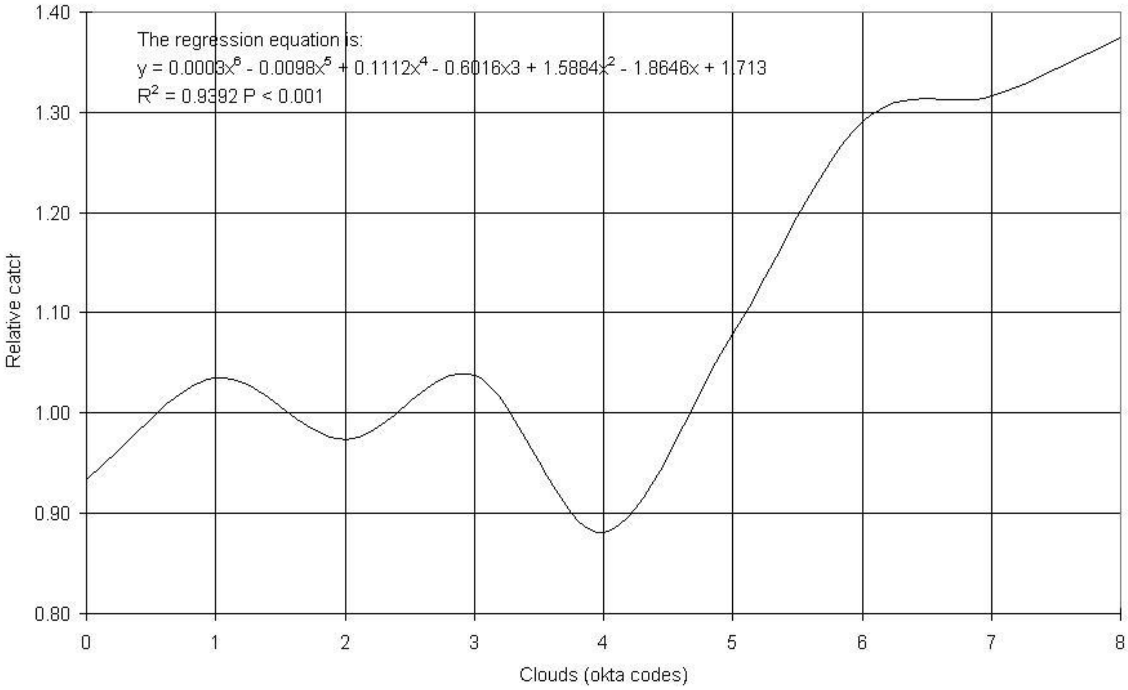

Figure 3. *Light-trap catch of the Turnip Moth (Agrotis segetum Den. et Schiff.) depending on the clouds in moonless hours (Kecskemét, 1967-1969)*

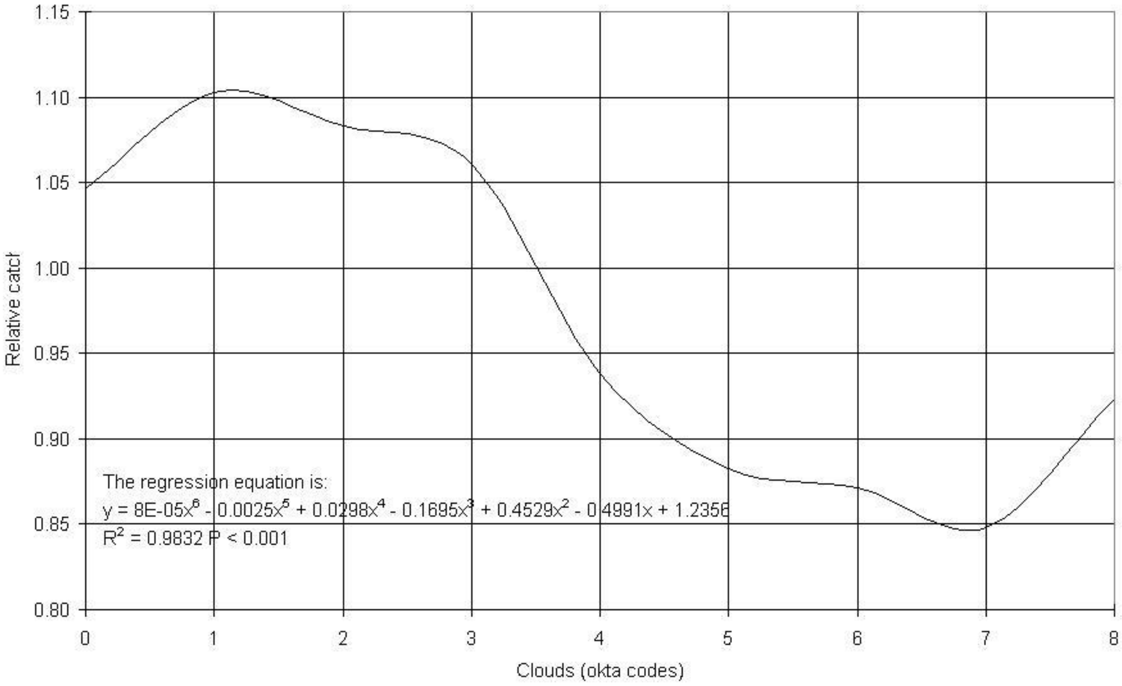

Figure 4. *The light-trap catch of the Turnip Moth (Agrotis segetum Den. et Schiff.) depending on the clouds in moonlit hours (Kecskemét, 1967-1969)*

The averaged numbers of individuals and species depending on the all clouds around the New Moon and Full Moon caught in Kámon Botanic Garden can be seen in *Fig. 5* and *Fig. 6*

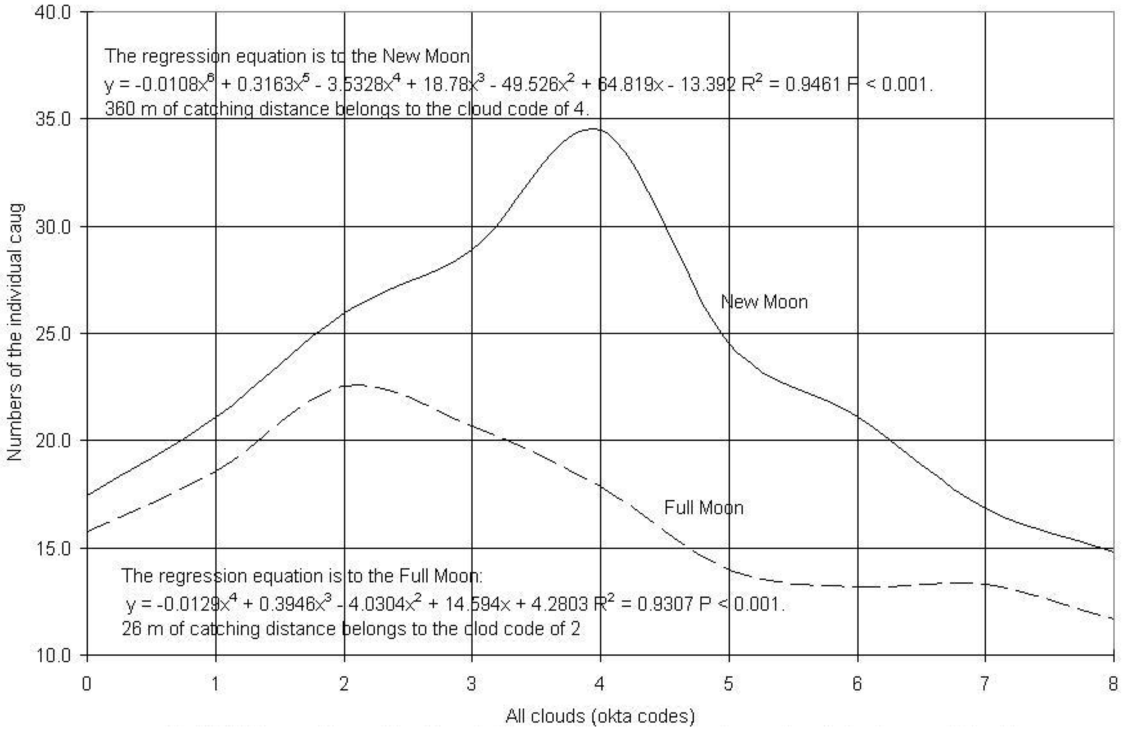

Figure 5. *Light-trap catches of the Macrolepidoptera individuals depending on the all clouds around New Moon and Full Moon in the Kámon Botanic Garden between 1962 and 1970*

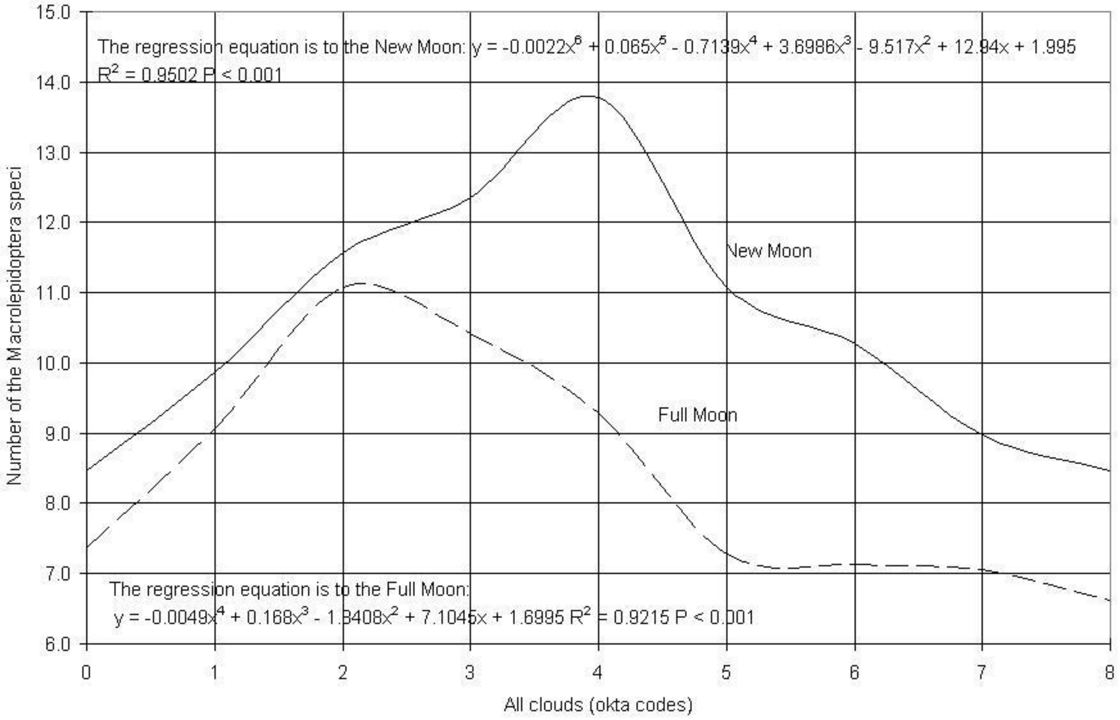

Figure 6. *Light-trap catches of the Macrolepidoptera* <u>*species*</u> *depending on the all clouds around New Moon and Full Moon int he Kámon Botanic Garden between 1962 and 1970*

Fig. 7 and *Fig 8* illustrate the catching results of the individuals and species in connection with the low clouds.

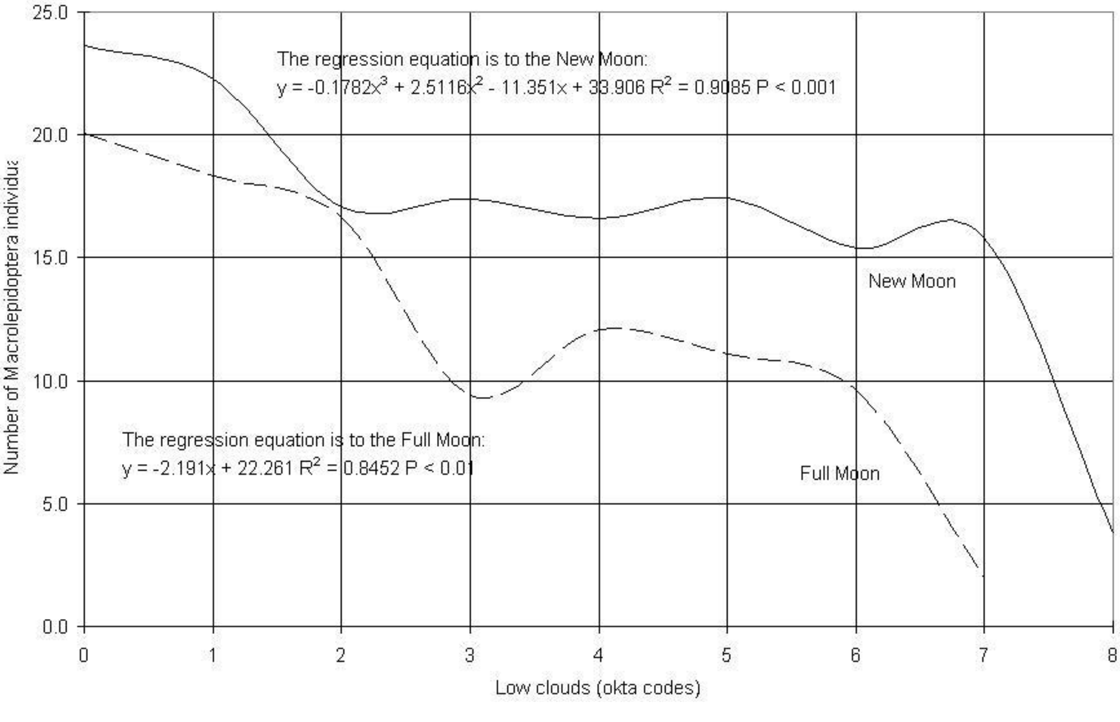

Figure 7. *Light-trap catches of the Macrolepidoptera* <u>*individuals*</u> *depending on the low clouds around New Moon and Full Moon in Kámon Botanic Garden between 1962 and 1970*

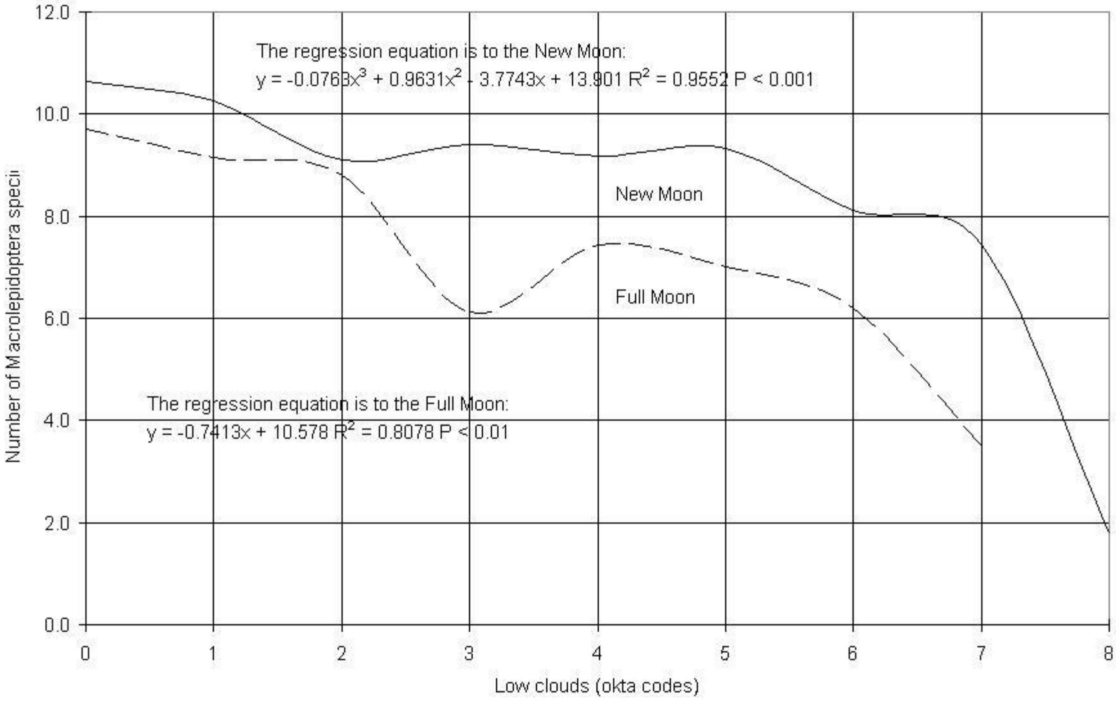

Figure 8. *Light-trap catches of the Macrolepidoptera* <u>*species*</u> *depending on the low clouds around New Moon and Full Moon in Kámon Botanic Garden between 1962 and 1970*

The *Table 1* and *Table 2* imply the light trapping individuals' and species' number in the function of the cloud cover, the four quarters of the Moon.

Table 1. *Number of Macrolepidoptera* <u>*individuals*</u> *caught by light-trap in Kámon Botanic Garden in Szombathely between 1962 and 1970 depending on the moon phases and clouds*

Clouds	*Clear (0-2)*		*Intermediate (3-5)*		*Cloudy (6-8)*	
Moon phases	*Individuals*	*Data*	*Individuals*	*Data*	*Individuals*	*Data*
New Moon	24.93	196	19.49	243	18.66	135
First Quarter	29.48	118	17.94	135	13.37	87
Full Moon	21.97	89	18.02	104	12.86	63
Last Quarter	26.91	117	16.97	162	24.18	80

Notes: Data = Number of observing data. Significance levels: New Moon: Intermediate – Cloudy $P < 0.05$; First Quarter: Clear – Intermediate $P < 0.01$, Clear – Cloudy $P < 0.01$; Full Moon: Clear– Cloudy $P < 0.05$; Last Quarter: Clear – Intermediate $P < 0.01$, Intermediate – Cloudy $P < 0.05$.

Table 2. *Number of Macrolepidoptera* <u>*species*</u> *caught by light-trap in Kámon Botanic Garden in Szombathely between 1962 and 1970 depending on the moon phases and clouds*

Clouds	*Clear (0-2)*		*Intermediate (3-5)*		*Cloudy (6-8)*	
Moon phases	*Species*	*Data*	*Species*	*Data*	*Species*	*Data*
New Moon	11.96	196	9.74	243	9.35	135
First Quarter	13.09	118	9.27	135	7.47	87
Full Moon	10.61	89	9.19	104	6.60	63
Last Quarter	13.39	117	9.42	162	10.79	80

Notes: Data = Number of observing data. Significance levels: New Moon: Intermediate – Cloudy $P < 0.01$; First Quarter: Clear – Intermediate $P < 0.01$, Clear – Cloudy $P < 0.01$; Full Moon: Intermediate – Cloudy $P < 0.05$; Last Quarter: Clear – Intermediate $P < 0.01$.

The *Fig. 9* and *Fig.10* show the modifying influence of the height of cloud base for the light-trap catch around the New Moon and Full Moon.

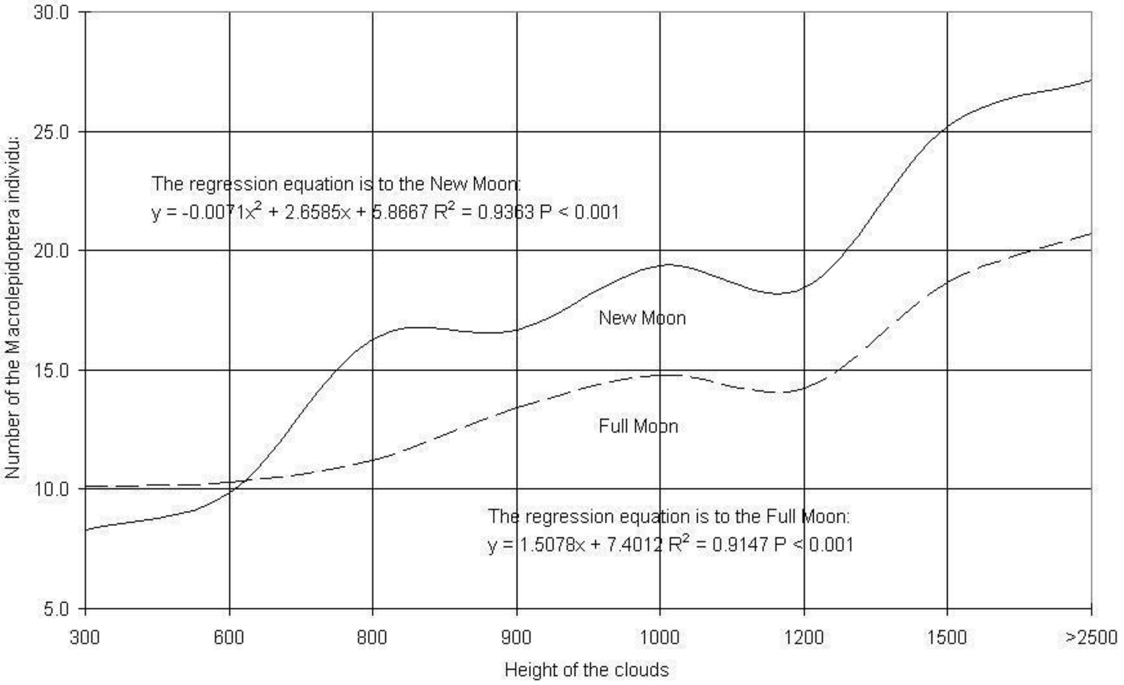

***Figure 9.** Light-trap catches of the Macrolepidoptera individuals depending on the height of clouds around New Moon and Full Moon in Kámon Botanic Garden between 1962 and 1970*

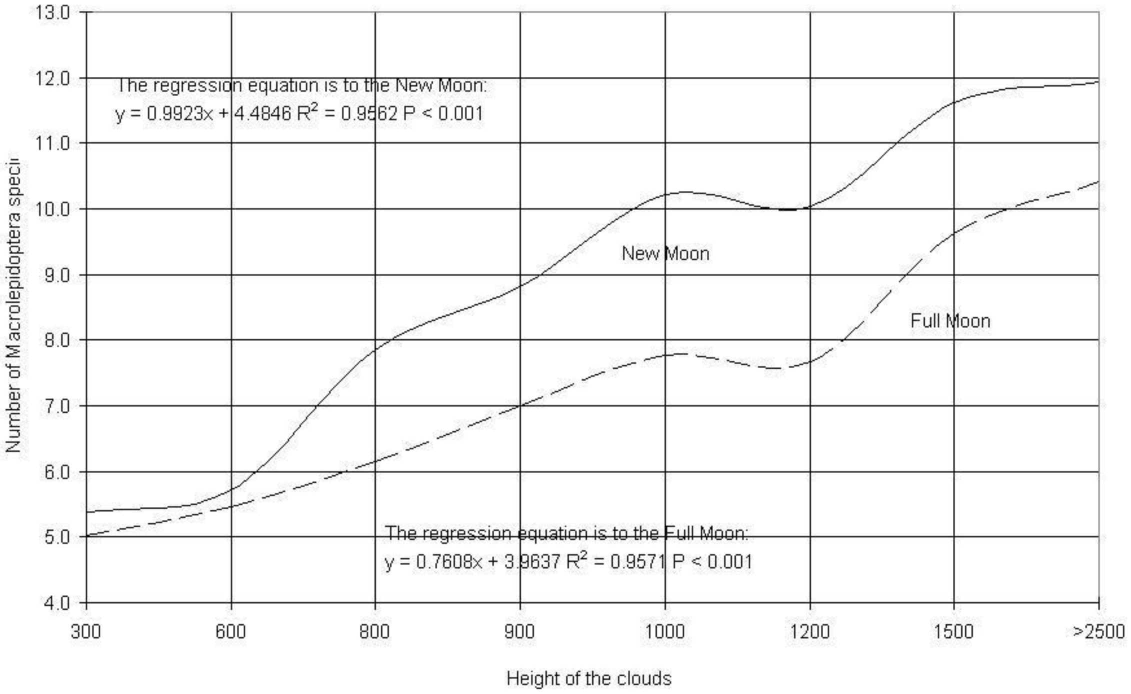

***Figure 10.** Light-trap catches of the Macrolepidoptera species depending on the height of clouds around New Moon and Full Moon in Kámon Botanic Garden between 1962 and 1970*

Discussion

The clouds determine the theoretical catching distances of both the Járfás and Jermy type light-traps fundamentally. The ratio of theoretical catching distances of completely overcast and clear sky is approximately 2.4:1. This difference does not appear however in the catching results.

It can be verified by our examinations that the catching of Turnip Moth (*Agrotis segetum* Den. et Schiff.) in moonless hours is the most successful (RC = 1.374) when the sky is totally through, if it is not raining (*Fig. 3*). However, if there is rain, the relative catch is only 0.518. The difference is significant at P < 0001.

In opposition to this, the catch decreases by the increase of the cloud cover in moonlit hours (*Fig. 4*). The most moths were found in the light-trap when the sky was almost clear. The increase of cloud cover results in a reduction of the catch.

The explanation for this phenomenon might be that the moon will help in the insects in orientation (Nowinszky, 2008). Notice that moonlit hours have been demonstrated catches associated with fully cloud. This case occurs when the sky is clear over a part of the hour; the Moon appears, however, in different part of the hour, the sky is completely overcast, but rain does not fall.

The results shown in *Fig. 5-8* also demonstrates that the number of the Macrolepidoptera individuals and species are higher when the sky is clear than overcast in the event both the all and low clouds.

Table 1 and *2* clearly show that the individuals and species caught by the Jermy type light-traps in all four moon phases are higher in bright than the overcast skies.

It was also found that the height of cloud base also modify the light-trap catch. If the cloud base is low the light-trap catch is also low, in the opposite it is high (*Fig. 9* and *Fig. 10*).

Our examinations justify that the behaviour of single Macrolepidoptera species may be different naturally, onto the catch of most species more successful the moonlit and bright nights. Our results contradict Williams (1936) and for the results of more other researchers. In our opinion the overcast sky increased of a catching distance before with decades the number of the individuals caught may have been higher really. We deduce from our own results that the reducing effect of catching distance of the moonlight may have prevailed limitedly only because of the vegetation in the botanic garden however. Since it is the botanic garden on the city's area though, from the centre some 2 km extends, the urban public lighting, the neon signs and the light seeping out from the flats caused light pollution although this concept was not used yet then.

The collection distance was not significantly bigger at the time of New Moon because of this, than at the time of a Full Moon. The orientation of the moths happened based on light stimuli when the clouds did not cover the moon though; this fact increased the efficiency of the trapping. Baker (1987) and Dacke et al. (2003) proved that certain moths and beetles use for their orientation the Moon and polarized moonlight.

The catching results of a fractionating light-trap confirm the species examined orient based on light stimuli in moonlit hours and bright sky. The catch is higher because the reducing effect of a smaller catching distance with a smaller effect at this time. The longer catching distance prevailed in moonless hours for the Turnip Moth (*Agrotis segetum* Den. et Schiff.) In the our studies appeared recent past we established that light pollution grew in the latter decades impugn the influence of the changing of catching distance on the light trapping result (Nowinszky, 2006 and 2008).

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INFLUENCE OF TILLAGE TECHNIQUES AND ORGANIC MATTER ON CARBON AND NITROGEN TRANSFORMATION IN THE RICE RHIZOSPHERE IN AN ALLUVIAL SOIL OF WEST BENGAL

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Abstract. The investigation highlights results of a field experiment conducted in an Inceptisol in the Lower Gangetic Plain of India to evaluate the sole and interactive effect of different degree of puddling through various tillage implements and farm yard manure (FYM) on carbon and nitrogen transformation in the rice rhizosphere over two seasons. Three methods of puddling viz., (1) Light puddling with country plough, (2) Moderate puddling with tractor drawn cultivator and (3) Intensive puddling with power tiller, were employed in specified triplicate plots with and without application of FYM and their effect on organic carbon, microbial biomass carbon, NH_4^+-N, NO_3^--N and total nitrogen in the rice rhizosphere soil were assessed. The results of this study revealed that while light puddling induced the highest build up of soil organic carbon, microbial biomass carbon and total nitrogen. Moderate puddling effectuated the highest level of NH_4^+-N and NO_3^--N suggesting the highest rate of mineralization of organic-N in rice rhizosphere. Irrespective of puddling intensity, FYM escalated its positive effects. The highest level of NO_3^- - N was induced through the rhizosphere during flowering stage. All the chemical and biochemical attributes of the soil, except NO_3^- N, were stimulated to the greatest extent during tillering stage. From this research results moderate puddling could be suggested for the best nitrogen availability during the critical growth stages of rice.

Keywords: *Tillage, Organic matter, N transformation, rice, rhizosphere*

Introduction

Rice has always played a very important role in India's food security and civilization and is the staple food for more than 2.5 billion people in Asia and many more millions in Africa and Latin America. To feed the increasing global population, annual production of rice must increase from the present level to at least 760 Mt by the year 2020 (Kundu and Ladha, 1995). The future of the food security system, not only in India but the whole of Asia, banks upon the ability to achieve a trend of growth towards productivity and profitability of rice farming systems on an ecologically sustainable basis (Swaminathan, 1993). However, the recent trend since early 1980s has been showing downward compound growth rate in area, production and productivity of rice (Taimani and Verma, 1998). Yield stagnation and even decline in rice yield in Asia are commonly associated with gradual decline in the supply of soil nutrients especially nitrogen (Flinn and De Datta, 1984) and soil organic carbon (Cassman et al., 1995; Duxbury et al., 2000; Yadav et al., 1998; 2000). Though the role of fertilizer N in enhancing N mineralization rate in soil (Bouldin, 1986; Broadbent, 1979) and soil N uptake by wetland rice (De Datta and Broadbent, 1990) is well understood, data obtained from several field experiments under diverse soil and climatic conditions suggest that a major part ranging from 56-93% of the total N-requirement of wetland rice crop even when supplied with adequate fertilizer N particularly during heading to maturity stage (Wada et al., 1986) is met from soil organic N source (Broadbent, 1979). So, different management approaches must be evolved to explore this otherwise biggest N source for economical use of N-fertilizer.

The quality of cultivated soil is affected by various agricultural practices and among them tillage holds an important place. It is important that the soil management system provide agricultural sustainability and minimal detrimental effect on the environment. Puddling, the mechanical manipulation of soil, is important for making soil condition favorable for transplanting of rice seedlings. But puddling exposes soil aggregates and results in destruction of stable soil aggregates leading to microbial decomposition of the exposed clay-organic matter complexes and, thus, causes loss of soil organic matter. The fate and behavior of soil organic matter are regulated by the degree of intensity of soil tillage. When crop residues remain on the surface, the oxidation rate of organic matter is reduced and soil organic matter accumulates on the soil surface (Beare et al., 1994). Intensive tillage like puddling, loosen and invert soil and makes soil more friable and allows greater oxygen diffusion resulting in higher activity of aerobic micro flora which, in turn, effectuate a rapid loss of CO_2 form soil (Reicosky and Lindstrom, 1995). Moreover, puddling exposes higher surface area of soil and so greater interaction among inanimate constituents including minerals as well as organics in soil and biotic constituents is achieved. This in turn, effectuates enhancement in aerobic microbial decomposition of incorporated residues resulting in a net decrease in soil organic carbon (Reicosky and Lindstrom, 1995). Management system based on reduction of soil disturbance and tillage depth leads to an accumulation of organic matter (Campbell et al., 1998; Costantini et al., 1996) and enrichment of soil N and soil P (Diaz-Zorita and Grove, 2002; Zibilske et al., 2002).

Soil organic carbon is an important index of soil quality and so plays a crucial role in sustaining crop productivity and environmental quality (Campbell et al., 1996; Doran, 1980; Lal et al., 1997) by rejuvenating soil physical, chemical and biological properties viz., soil water retention and availability, nutrient cycling, gas flux, plant root growth and soil conservation (Gregorich, 1994). Soil microorganisms play integral role in nutrient cycling, soil stabilization and organic matter decomposition. Greater soil disturbance decrease average microbial biomass (Follett and Schimel, 1989) while minimum tillage practice provides means to reduce loss of carbon (Morris et al., 2004). So, appropriate land use and soil management practices make a dent for the improvement in soil organic carbon and so soil quality besides mitigating partially the rise of atmospheric CO_2 (Lal and Bruce, 1999; Paustian et al., 1997). Among the carbon pools in soil, soil microbial biomass carbon is the only living entity. It quickly interacts and manifests status of soil health well in advance as microbes are closely associated with soil matrix. Thus, the effect of puddling intensity on rice rhizosphere can be assessed through this tool.

Incorporated organic matter in soil holds a huge potential to mitigate the adverse effects of tillage to a great extent by improving soil microbial biomass pool (Doran, 1980), carbon, nitrogen and other nutrient transformation (Campbell et al., 1998) through improvement of enzymatic activities (Flinn and De Datta, 1984). Proper puddling of rice soils in combination with organic matter management may lead to improvement of rice crop growth, maintain proper ecology of the rhizosphere and may ultimately lead to higher production- both quantitatively and qualitatively, on a sustainable basis.

The present research project was undertaken to study the influence of different puddling practices varying in the intensity and depth of ploughing practices in combination with farm yard manure on soil organic carbon and nitrogen transformation in an alluvial (Inceptisol) soil of Eastern India.

Materials and methods

Site and soil information

The investigation comprises two field experiments in succession during *kharif* (Rainy; July-October), 2003 and *rabi* (pre-summer; January-April) season of 2004 at the Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya at Gayespur located at 22°58' N and 88°31' E at an elevation of 9.75 m above mean sea level (MSL). The Research Farm is situated in the sub-humid zone of eastern India. Soil of the experimental site is classified as fine loamy Aeric Haplaquept. Some of the basic chemical, physico-chemical and bio-chemical properties of the surface (0-15 cm) soil are presented in *Table 1*.

Table 1. *Some important characteristics of the experimental soil*

Parameters	**Values**
Textural class	Sandy loam
Sand (%)	51.7
Silt (%)	17.1
Clay (%)	17.1
pH (1:2.5 Soil: Water ratio)	7.45
Electrical conductivity (dS m^{-1})	1.916
CEC [C.mole (p^+).kg^{-1}]	12.8
Organic carbon (g.kg^{-1})	4.80
Total N (g.kg^{-1})	0.47
Ammonium – N (kg.ha^{-1})	130.16
Nitrate – N (kg.ha^{-1})	127.32
Available P (kg.ha^{-1})	9.70
Available K (kg.ha^{-1})	128.27
Microbial biomass carbon (µg.g^{-1})	28.65

Field investigation

Three puddling methods typical to the region were implemented: conventional light, moderate and intense puddling. Conventional light puddling, to a depth of 0-50 mm, was accomplished by an indigenous plough made of wood with an iron soil cutting edge which causes no inversion (Kamble and Srivastava, 2003), while moderate puddling, to a depth of 0-100 mm, was accomplished by using a spring tine cultivator drawn by a tractor fitted with cage wheels and intense puddling to a depth of 0-150 mm, was accomplished by a rotary power tiller. Surface leveling was done by a horizontal wooden beam (indigenous leveler). Factorial combination of these tillage methods and 2 levels of organic manure viz., (1) No Organic Manure (F_1) and (2) Farm Yard Manure 7.5 t.ha^{-1} (F_2), were applied in (10m × 8m) triplicate plots in a Randomized Complete Block Design. Three weeks old healthy seedlings of rice variety *Satabdi* at 3-4 seedlings.hill^{-1} were transplanted at 25 cm x 10 cm spacing. Fertilizer nitrogen 80 kg.ha^{-1} as urea, phosphorus 60 kg.ha^{-1} as single super phosphate and potassium 40 kg.ha^{-1} as muriate of potash were applied to each plot. While phosphorus and potassium were applied at a time as basal dressing during final land preparation, nitrogen was applied in three equal splits. Irrigation, intercultural operations including pest and disease management were done as and when necessary. The crop was grown to maturity.

Collection and analyses of soil samples

Initial bulk surface (0-150 mm) soil samples were collected from field before the start of the experiment. Soil samples from each plot were also collected during critical growth periods like: tillering, flowering and maturity. The soil samples were analyzed for pH in 1:2.5 soil: water suspension (Jackson, 1973), oxidisable soil organic carbon by the method of Walkley and Black (1934), total nitrogen by wet digestion and Kjeldahl distillation method (Jackson, 1973), ammonium and nitrate nitrogen after extraction with KCl solution and Kjeldahl distillation method using Devarda’s alloy for nitrate (Jackson, 1973), available P following the method of Olsen et al. (1954), available K by 1N NH_4OAC using a flame photometer (Jackson, 1973) and microbial biomass carbon by method described by Joergenson (1995). Core sampler was used for soil bulk density determination. Soil texture was determined by Bouyoucos hydrometer (Bouyoucos, 1962). Soil samples collected during different stages of rice growth were also analyzed for oxidisable soil organic carbon, total nitrogen, ammonium and nitrate nitrogen and microbial biomass carbon following standard methods described above.

Statistical analysis

Statistical significance of treatment effects on different properties of soil was inferred from least significant difference (LSD, P=0.05) test using analysis of variance from a Randomized Complete Block Design.

Results and discussion

Organic Carbon

Perusal of results (*Table 2*) reveal that conventional light puddling with country plough caused a significant increase in the organic carbon level, as compared to the other puddling methods, in the rice rhizosphere during each of the growth stages. The impact was further accelerated by the applied FYM to the soil. The increase in the organic carbon level of rhizosphere soil was due to less exposure of soil particles resulting in lower availability of autochthonous micro-flora on soil organic matter. The result substantiated the report of Doran (1980).

***Table 2.** Effect of puddling practices and farm yard manure (FYM) on soil rhizosphere organic carbon status (g.kg^{-1}) at different crop growth stages during two rice growing seasons*

Puddling Practices	Crop Growth Stages											
	Maximum Tillering			Flowering			Maturity			Mean		
	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean
Kharif, 2003:												
Light Puddling	5.97	5.42	5.69	5.70	5.20	5.45	5.50	5.10	5.30	5.72	5.24	5.48
Moderate Puddling	5.70	5.20	5.45	5.50	5.03	5.27	5.15	4.85	5.00	5.45	5.03	5.24
Intense Puddling	5.88	5.32	5.60	5.63	5.17	5.40	5.40	4.90	5.15	5.64	5.13	5.38
Mean	5.85	5.31	5.58	5.61	5.13	5.37	5.35	4.95	5.15	5.60	5.13	
LSD (P=0.05)	Growth stage: 0.098 ; Puddling: 0.098; FYM: 0.080; Growth stage x Puddling: 0.170; Puddling x FYM: 0.139; Growth stage x FYM: 0.139											
Rabi, 2004:												
Light Puddling	6.13	5.60	5.87	5.80	5.30	5.55	5.63	5.20	5.42	5.85	5.37	5.61
Moderate Puddling	5.80	5.25	5.52	5.75	5.23	5.49	5.25	4.90	5.08	5.60	5.13	5.36
Intense Puddling	5.96	5.36	5.66	5.76	5.26	5.51	5.53	5.16	5.35	5.75	5.26	5.51
Mean	5.95	5.40	5.68	5.79	5.26	5.53	5.47	5.09	5.28	5.74	5.25	5.49
LSD (P=0.05)	Growth stage: 0.074 ; Puddling: 0.074; FYM: 0.061; Growth stage x Puddling: 0.129; Puddling x FYM: 0.105; Growth stage x FYM: 0.105											

Addition of FYM resulted in a significant increase in the organic carbon level of rhizosphere soil from tillering to maturity stage of rice over that of FYM untreated plots, which was due to the additive effect of carbonaceous compounds of FYM (Mukherjee and Gaur, 1984). The organic carbon content of rhizosphere soil significantly decreased from tillering to maturity stage of rice due to greater utilization of organic carbonaceous materials by the increased number of micro-organisms resulting in higher breakdown of organic matter with a subsequent release of CO_2 from the soil (Debnath et al., 1994). Organic carbon was positively and significantly correlated with microbial biomass carbon, total nitrogen content, ammonium nitrogen content and nitrate nitrogen content in the rice rhizosphere (*Table 7*).

Total Nitrogen

Compared to other puddling methods, conventional light puddling with country plough effectuated significant increase in the total –N content of rhizosphere during all the growth stages of rice (*Table 3*). The increase was further accelerated by the addition of FYM in soil. Light puddling resulted in less exposure of the soil particles leading to less proliferation of microorganisms. This in turn brought about lower mineralization of organic nitrogenous compounds protected in the lattice holes of minerals in the soil of rhizosphere.

Addition of FYM exerted significant increase in the total-N content of rhizosphere soil during each of the growth stages due to additive effect of nitrogenous components of the organic manure (Saenjan et al., 1992). Moreover, added FYM effectuated assymbiotic nitrogen fixation by free living aerobic and anaerobic N-fixers in rice rhizophere. The level of total-N significantly decreased from tillering to maturity stage in the rhizosphere soil due to uptake of available N by the rice crop or loss of N through biotic and abiotic processes after mineralization of organic nitrogenous compounds in the rhizosphere of rice. More so, the capacity of free living N-fixers to fix atmospheric nitrogen decreased with the advancement of the age of plants due to decreased rate of rhizo-deposition (Saha, 2000). Total-N content was significantly correlated with microbial biomass carbon, organic carbon, ammonium nitrogen content and nitrate nitrogen content in the rice rhizosphere (*Table 7*).

Table 3. *Effect of puddling practices and farm yard manure (FYM) on soil rhizosphere total nitrogen status (g.kg^{-1}) at different crop growth stages during two rice growing seasons*

Puddling Practices	Crop Growth Stages											
	Maximum Tillering			Flowering			Maturity			Mean		
					Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean
Kharif, 2003:												
Light Puddling	0.59	0.53	0.56	0.57	0.51	0.54	0.53	0.49	0.51	0.56	0.51	0.54
Moderate Puddling	0.55	0.50	0.53	0.51	0.48	0.50	0.48	0.46	0.47	0.51	0.48	0.50
Intense Puddling	0.56	0.51	0.54	0.53	0.49	0.51	0.50	0.47	0.49	0.53	0.49	0.51
Mean	0.57	0.51	0.54	0.54	0.49	0.52	0.50	0.47	0.49	0.54	0.49	
LSD (P=0.05)	Growth stage: 0.010 ; Puddling: 0.010; FYM: 0.008; Growth stage x Puddling: 0.017; Puddling x FYM: 0.014; Growth stage x FYM: 0.014											
Rabi, 2004:												
Light Puddling	0.61	0.54	0.58	0.58	0.52	0.55	0.55	0.50	0.53	0.58	0.52	0.55
Moderate Puddling	0.57	0.52	0.55	0.54	0.50	0.52	0.51	0.48	0.50	0.54	0.50	0.52
Intense Puddling	0.59	0.53	0.56	0.56	0.52	0.54	0.53	0.49	0.51	0.56	0.51	0.54
Mean	0.59	0.53	0.56	0.56	0.51	0.54	0.53	0.49	0.51	0.56	0.51	0.54
LSD (P=0.05)	Growth stage: 0.010 ; Puddling: 0.010; FYM: 0.008; Growth stage x Puddling: 0.017; Puddling x FYM: 0.014; Growth stage x FYM: 0.014											

Ammonium and Nitrate Nitrogen

Among different puddling methods, while moderate puddling by tractor drawn cultivator resulted in the highest level of both ammonium and nitrate nitrogen in the rhizosphere soil at different growth stages of rice, the light puddling by country plough resulted in the lowest status (*Table 4, 5*).

Table 4. *Effect of puddling practices and farm yard manure (FYM) on soil rhizosphere ammoniacal nitrogen status (kg.ha^{-1}) at different crop growth stages during two rice growing seasons*

Puddling Practices	Crop Growth Stages											
	Maximum Tillering			Flowering			Maturity			Mean		
	With FYM	Without FYM	Mean	With FYM	Without FYM	Mean	With FYM	Without FYM	Mean	With FYM	Without FYM	Mean
Kharif, 2003:												
Light Puddling	150.40	135.20	142.80	141.43	131.50	136.47	135.40	129.30	132.35	142.41	132.00	137.21
Moderate Puddling	165.50	142.47	153.98	156.27	137.40	146.83	145.30	133.73	139.52	155.69	137.87	146.78
Intense Puddling	158.60	138.60	148.60	148.53	133.40	140.97	139.17	131.43	135.30	148.77	134.48	141.62
Mean	158.17	138.76	148.46	148.74	134.10	141.42	139.96	131.49	135.72	148.96	134.78	
LSD (P=0.05)	Growth stage:0.104 ; Puddling:0.104; FYM:0.085; Growth stage x Puddling:0.181; Puddling x FYM:0.148; Growth stage x FYM: 0.148											
Rabi, 2004:												
Light Puddling	154.63	137.50	146.07	146.50	137.24	131.33	140.38	135.40	139.47	146.87	134.37	140.62
Moderate Puddling	167.27	143.27	155.27	161.30	140.27	135.47	150.78	143.60	151.73	160.10	139.67	149.88
Intense Puddling	160.27	139.50	149.88	151.30	135.40	132.60	145.35	138.72	144.83	152.13	135.83	143.98
Mean	160.72	144.09	150.41	153.03	136.64	133.13	144.84	139.24	145.34	153.03	136.62	144.83
LSD (P=0.05)	Growth stage: 0.116 ; Puddling: 0.116; FYM: 0.094; Growth stage x Puddling: 0.200; Puddling x FYM: 0.163; Growth stage x FYM: 0.163											

Addition of FYM, in general, improved the ammonium and nitrate nitrogen in the rhizosphere soil compared to the FYM untreated control soil. While the ammonium-N content of rhizosphere soil was highest during tillering stage, the nitrate-N content was the highest during flowering stage and that reflected the higher rate of nitrification during flowering stage of rice. Among different puddling methods, moderate puddling with tractor drawn cultivator resulted in the highest exposure of primary and secondary soil particles and furnished favorable conditions for microbial proliferation leading to greater mineralization of both native soil organic matter as well as added organic nitrogenous compounds.

Table 5. *Effect of puddling practices and farm yard manure (FYM) on soil rhizosphere nitrate nitrogen status (kg.ha^{-1}) at different crop growth stages during two rice growing seasons*

Puddling Practices	Crop Growth Stages											
	Maximum Tillering			Flowering			Maturity			Mean		
	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean
Kharif, 2003:												
Light Puddling	148.70	134.53	141.62	158.40	140.30	149.35	145.23	132.50	138.87	150.78	135.78	143.28
Moderate Puddling	157.80	140.80	149.30	166.40	146.20	156.30	153.40	137.23	145.32	159.20	141.41	150.31
Intense Puddling	153.40	136.23	144.82	161.23	143.40	152.32	149.30	134.20	141.75	154.64	137.94	146.29
Mean	153.30	137.19	145.24	162.01	143.30	152.66	149.31	134.64	141.98	154.87	138.38	
LSD (P=0.05)	Growth stage: 0.094 ; Puddling: 0.094; FYM: 0.077; Growth stage x Puddling: 0.163; Puddling x FYM: 0.133; Growth stage x FYM: 0.133											
Rabi, 2004:												
Light Puddling	150.30	136.00	143.15	157.30	141.40	149.35	148.40	134.40	141.40	152.00	137.27	144.63
Moderate Puddling	163.67	142.00	152.83	169.30	146.23	157.77	160.20	140.30	150.25	164.39	142.84	153.62
Intense Puddling	157.80	138.40	148.10	166.43	145.30	155.87	157.50	136.20	146.85	160.58	139.97	150.27
Mean	157.26	138.80	148.03	164.34	144.31	154.33	155.37	136.97	146.17	158.99	140.03	149.51
LSD (P=0.05)	Growth stage: 0.163 ; Puddling: 0.163; FYM: 0.133; Growth stage x Puddling: 0.283; Puddling x FYM: 0.231; Growth stage x FYM: 0.231											

Higher amounts of ammonium and nitrate nitrogen in FYM treated soil were due to the additive effects of the mineralized organic nitrogen compounds of the organic manure (Mukherjee and Gaur, 1985). The highest level of ammonium-N at the tillering stage was due to greater rate of ammonification of organic manure (Mishra et al., 2001). Ammonium-N of the rhizosphere was significantly and positively correlated with microbial biomass carbon, organic carbon, total nitrogen content and nitrate nitrogen content in the rice rhizosphere (*Table 7*). The nitrate-N content of rice rhizosphere was significantly and positively correlated with microbial biomass carbon, organic carbon, ammonium nitrogen content and total nitrogen content (*Table 7*).

Microbial Biomass Carbon

Among different puddling methods, while conventional light puddling with country plough resulted in the highest level, moderate puddling with tractor drawn cultivator caused the lowest level of microbial biomass carbon in the rice rhizosphere throughout all the growth stages of rice *(Table 6).* The increment in the level of microbial biomass carbon was due to the impact of higher level of organic carbon *(Table 2).* The result substantiates the reports of Spieir et al. (1982). It is globally hypothesized that density of microbial population declines with the intensity of agricultural operation (Giller et al., 1997; Pankhurst et al., 1995). In the present investigation, a gradient of intensity of disturbance created by puddling existed. As in case of conventional light puddling with country plough, the disturbance was relatively low, and thus, microbial habitat was relatively protected. This resulted in higher microbial loading which in turn, effectuated higher level of microbial biomass carbon. Application of FYM effectuated a universal scaling up of microbial biomass carbon in rice rhizosphere. The results, thus, corroborated the findings of (Bhattacharjee et al., 2001). The level of microbial biomass carbon gradually reduced from tillering to maturity stage in the rice rhizosphere due to gradual loss of organic carbon during those periods as evidenced from *Table 2*. It has been estimated that as much as 40% of plant primary production may be lost through rhizodeposition (Lynch and Whipps, 1990) in the form of very essential vitamins, short chain fatty acids, carbohydrates, amino acids etc., for microbial growth. But with advancement of growth of rice, quality and quantity of rhizodeposition decreased due to senescence (Hütsch et al., 2002). Thus, abundance of microbial population, in general and microbial biomass, in particular, declined.

Table 6. *Effect of puddling practices and farm yard manure (FYM) on soil rhizosphere microbial biomass carbon status ($\mu g.g^{-1}$) at different crop growth stages during two rice growing seasons*

Puddling Practices	Crop Growth Stages											
	Maximum Tillering			Flowering			Maturity			Mean		
	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean	With FYM	Without Organic Manure	Mean
Kharif, 2003:												
Light Puddling	103.30	49.37	76.33	96.43	46.30	71.37	84.83	40.57	62.70	94.86	45.41	70.13
Moderate Puddling	98.97	44.73	71.85	92.10	41.63	66.87	80.20	35.43	57.82	90.42	40.60	65.51
Intense Puddling	101.93	46.97	74.45	94.30	43.27	68.78	82.27	37.50	59.88	92.83	42.58	67.71
Mean	101.40	47.02	74.21	94.28	43.73	69.01	82.43	37.83	60.13	92.70	42.86	
LSD (P=0.05)	Growth stage: 0.397 ; Puddling: 0.397; FYM: 0.324; Growth stage x Puddling: 0.688; Puddling x FYM: 0.561; Growth stage x FYM: 0.561											
Light Puddling	116.83	54.30	85.57	110.60	47.30	78.95	96.63	42.77	69.70	108.02	48.12	78.07
Moderate Puddling	111.80	50.40	81.10	102.70	43.37	73.03	92.17	38.73	65.45	102.22	44.17	73.19
Intense Puddling	113.43	51.60	82.52	105.37	45.83	75.60	93.97	40.07	67.02	104.26	45.83	75.05
Mean	114.02	52.1o	83.06	106.22	45.50	75.86	94.26	40.52	67.39	104.83	46.04	75.44
LSD (P=0.05)	Growth stage: 0.883 ; Puddling: 0.883; FYM: 0.721; Growth stage x Puddling: 1.530; Puddling x FYM: 1.249; Growth stage x FYM: 1.249											

This is supported by the findings of Butler et al. (2004). With 14_C labeled carbon the authors showed that the turnover of labeled rhizodeposition through dynamics of microbial biomass was different in different phenological stages of crop growth. Microbial biomass carbon level of the rice rhizosphere was significantly positively correlated with organic carbon, total nitrogen, ammonium nitrogen and nitrate nitrogen content *(Table 7)*.

Table 7. *Pearson Correlation Co-efficient among different parameters of rhizosphere soil of rice during two growing seasons*

	Org. Carbon		Total N		Amm. N		Nitr. N		MBCarbon	
	Kharif,2003	Rabi, 2004	Kharif,2003	Rabi, 2004	Kharif,2003	Rabi, 2004	Kharif,2003	Rabi, 2004	Kharif,2003	Rabi, 2004
Tillering stage:										
Org. Carbon	1.00	1.00								
Total N	0.85**	0.89**	1.00	1.00						
Amm. N	0.66**	0.63**	0.53*	0.68**	1.00	1.00				
Nitr. N	0.71**	0.59**	0.59*	0.64**	0.99**	1.00**	1.00	1.00		
MBCarbon	0.93**	0.87**	0.82**	0.90**	0.86**	0.91**	0.90**	0.88**	1.00	1.00
Flowering stage:										
Org. Carbon	1.00	1.00								
Total N	0.76**	0.83**	1.00	1.00						
Amm. N	0.54*	0.81**	0.27	0.48*	1.00	1.00				
Nitr. N	0.68**	0.86**	0.49*	0.60*	0.96**	0.96**	1.00	1.00		
MBCarbon	0.80**	0.94**	0.75*	0.83**	0.81**	0.82**	0.93**	0.90**	1.00	1.00
Maturity stage:										
Org. Carbon	1.00	1.00								
Total N	0.76**	0.78**	1.00	1.00						
Amm. N	0.34	0.34	0.09	0.37	1.00	1.00				
Nitr. N	0.58*	0.47	0.33	0.48*	0.96**	0.98**	1.00	1.00		
MBCarbon	0.83**	0.75**	0.67*	0.73**	0.75**	0.82**	0.90**	0.89**	1.00	1.00

**Significant at 1% level of significance

*Significant at 5% level of significance

Conventional light puddling with country plough results in the lower microbial activity and so effectuates the highest build up of organic carbon, microbial biomass carbon but causes lower availability of plant available mineral nitrogen in the rhizosphere. Moderate puddling using tractor drawn cultivator, on the other hand, brings about higher mineralization of organic nitrogen and so causes an increment in the level of plant available mineral nitrogen in the rice rhizosphere. So, it is suggested that moderate puddling harnesses the best nitrogen availability in the rice rhizosphere in the critical growth stages, the impact of that is further accelerated through application of FYM.

Soil organic matter formation and development in rice fields is highly dependent on puddling intensity. Submergence makes added carbon recalcitrant and conserve for longer period while tillage withdraw the carbon balance. Thus, methods to maintain soil carbon level and aggressive tillage practices e.g. puddling are not compatible. Through further research knowledge to arrive at a delicate balance between applied organic residues and the intensity of puddling are needed for transplanting of rice seedlings and at the same time minimize agricultural impact on the environment.

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LONG-TERM DYNAMIC PATTERNS AND DIVERSITY OF PHYTOPLANKTON COMMUNITIES IN A LARGE EUTROPHIC RIVER (THE CASE OF RIVER DANUBE, HUNGARY)

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Abstract. In this paper we present the composition, seasonal dynamics and fluctuations in diversity of the phytoplankton in the Danube River over 24 years. Weekly samplings were conducted at one section of the river at Göd, in the 1669 river kilometer segment. The change in the phytoplankton community structure was analyzed in relation of water temperature and discharge means. Our findings support the opinion that the Danube is very rich in species, although many of the species are rare and could be described only as coloring species. Results indicate trends in the phytoplankton abundance, which are only detectable in long-term studies. By the help of diversity indices we have observed an increase in the phytoplankton community diversity. With the relevant information, an explanation of the significant changes in diversity and richness was formed. Our goals were a construction of a solid database of the phytoplankton, examining the seasonal dynamics of the phytoplankton through a 24 year long study and to see the most important changing factors of the community. The results of this study are to assist and help future model developments to predict the phytoplankton seasonal dynamic patterns.

Keywords: *river phytoplankton, LTER, diversity, multivariate analysis*

Introduction

During the last decades our knowledge about the taxonomical composition, quantitative relations, seasonal changes and long-term changes of phytoplankton in the Hungarian stretch of River Danube became more and more complete (Kiss, 1987; Kiss and Schmidt, 1998; Szemes, 1967a,b; Uherkovich, 1969). The phytoplankton is characterised by high densities of centric diatoms from early spring to late autumn. Besides the centrics, the chlorococcalean species are abundant during summer.

Because the nutrient supply of the river is high, the potential trophic level is hypertrophic or eutrophic (Déri, 1991; Varga et al., 1989). During the low water period 70-90 % of the water column belongs to the euphotic zone (Kiss, 1994). This is a favourable situation for the quick proliferation of the phytoplankton. The density of centric diatoms is 20000 – 70000 cell ml-1; biomass 10-30 mg l^{-1} at that time. This group forms 90-95 % of the abundance in spring and 65-75 % in summer. After floods, when the density of algae is low, they can develop quickly (Schmidt et al., 1996; Kiss, 1984; Kiss et al., 2006). The cell number of centrics can rise by two times during two-three days in summer (Kiss, 1994, 1996).

The phytoplankton abundance showed great seasonal and yearly changes (Kiss, 1994; Kiss and Schmidt, 1998; Vörös et al., 2000). Such results were presented about periphytic algae, too (Ács and Kiss, 1993) after comparing two years. Such detailed long term study of the phytoplankton of the River Danube was only performed for the Hungarian stretch. This current database could be the base of future river monitoring, for example (Ács et al., 2006). The importance of the long term scale is clearly shown in Sommer et al. (1993), since the small-scale change in weather for terrestrial vegetation has an effect of climatic magnitude for the phytoplankton, because of its fast reproduction rate. According to Reynolds (1997), one year change in the weather counts for a climatic change for the phytoplankton. Our study contributes to understand the community dynamics of the riverin phytoplankton (Tilman, 1982; Tilman et al., 1982) and provides valuable information for defending the community from harmful algal blooms (Anderson et al., 2008). Every phytoplankton study is of great importance, since the Earth phytoplankton biomass plays a capital role (compared to other living beings) in shaping the global climate (Charlson et al., 1987; Williamson and Gribbin, 1991). The understanding of long-term changes and seasonal dynamics is crucial for modelling. Our work serves as a base for the simulation modelling of seasonal dynamics (Sipkay et al., 2008a, 2008b) for which we already have strategic model of the theoretical algae community (Drégelyi-Kiss and Hufnagel, 2009a, 2009b).

The aim of this study was to construct a solid database of the phytoplankton, examining the seasonal dynamics of the phytoplankton through a 24 year long study and to see the most important changing factors of the community in a eutrophic large river.

Materials and methods

The River Danube is a eutrophic large river with relatively high water discharge (average water runoff at Budapest = 2300 m^3s^{-1}, average current velocity 1-1.5 ms^{-1}). Because of the turbulence and sampling method the phytoplankton is considered homogeneous, except for the areas of river bank. Samples were taken weekly sometimes biweekly between 1979 and 2002 upstream of Budapest, at Göd (1669 river kilometer), from near the surface of the river 5 times in 1 liter bottles with 1 minute intervals for Utermöhl method). This way a sample has been collected from a 500-600 meter long section, which homogeneousity is plausible (Kiss et al., 1996). Samples for phytoplankton analysis were fixed with Lugol solution. This study demonstrates data collected for 24 years, but sampling continues up to this day. The quantitative changes in the phytoplankton growth shows, that biweekly sampling is within an average of 10% margin of error (Kiss et al., 1996).

Quantitative analysis was performed using Utermöhl's method by inverted microscope (OPTON Invertoscope-D). For counting statistics and calculation errors suggestions of Lund et al. (1958) were used (400 algal specimens were counted, the calculation error is ±10 %). To get biomass value, the specimen number of each species needs to be multiplied with their peculiar cell volume. The outcome can uniformly be normalized to mg l^{-1} measure.

The examinations of species diversity that have a great specialized literature, the demand for the elaboration of species abundance models have emerged (Izsák and Szeidl, 2009), that is analyzing in what entity numbers (mass) do species take part in the examined community (Magurran, 1988; Pásztor and Oborny, 2007). The received gradation provides information about the power relations of the species.

The species abundance models start from the assumption that if a species (community) obtains bigger resources (nutriment, living space etc.), then it will appear in greater entity number (Izsák and Szeidl, 2009). Certain literature dealing with ecology mention three basic models: geometrical, lognormal, and broken stick models (Aoki, 1995; Dunbar et al., 2002; Kobayashi, 1985; Saldaña and Ibáñez; 2004; Skalskia and Pośpiechb, 2006). Beta-distribution is hardly mentioned by literature apart from a few exceptions (Fattorini, 2005; Muneepeerakula et al., 2007), we still use it, because in certain cases this model approximates abundance-distribution the best. Izsák and Szeidl (2009) verified that beta-distribution is very similar to lognormal distribution in case of certain parameters, and with the prosperous choice of parameter it nears well the broken stick model too.

Besides the conventional imagery and graphs, method of Gimesi (2008, 2009) of three-dimensional illustration was used to present annual and monthly trends on the same diagram. During the 24 years, 1176 samples were analysed and in the vicinity of 530 species were identified, thus Multivariate Data Analysis (such as Hierarchical Clustering and non-metric multidimensional scaling: “NMDS”) was used beside the conventional statistical analysis. In this analysis the spatial changes were left out of consideration, only temporal markings were studied. The datamatrix constructed from the sample data was also logarithmically transformed and binarized, in order to emphasize rare species in the analysis. Euclidean distance and the Past data analysis package ver. 1.36 and 1.55 were used for every multivariate analysis (Hammer et al., 2001).

Results

Long-term changes in phytoplankton abundance

During the investigated period of 1979-2002 a total of 528 taxa were identified from the phytoplankton at Göd. Compared to this number in 1998, 583 species were identified throughout the length of the river within Hungarian borders and 2696 algal taxa were mentioned in Kusel-Fetzmann’s summary (1998), which was published on the full length of the Danube. The most species belong to the classes of Chlorophyceae and Bacillarophyceae (*Fig. 1*). The abundance distribution was also much distorted. Although, the most abundant group, the Chlorophyceae class had 207 species, they only represented 5.8 % of the total biomass. The two Bacillarophyceae groups dominated the whole community with a 92.1 % participation and from that, only 28 Centrales taxa were responsible for 89.9 % of the phytoplankton biomass.

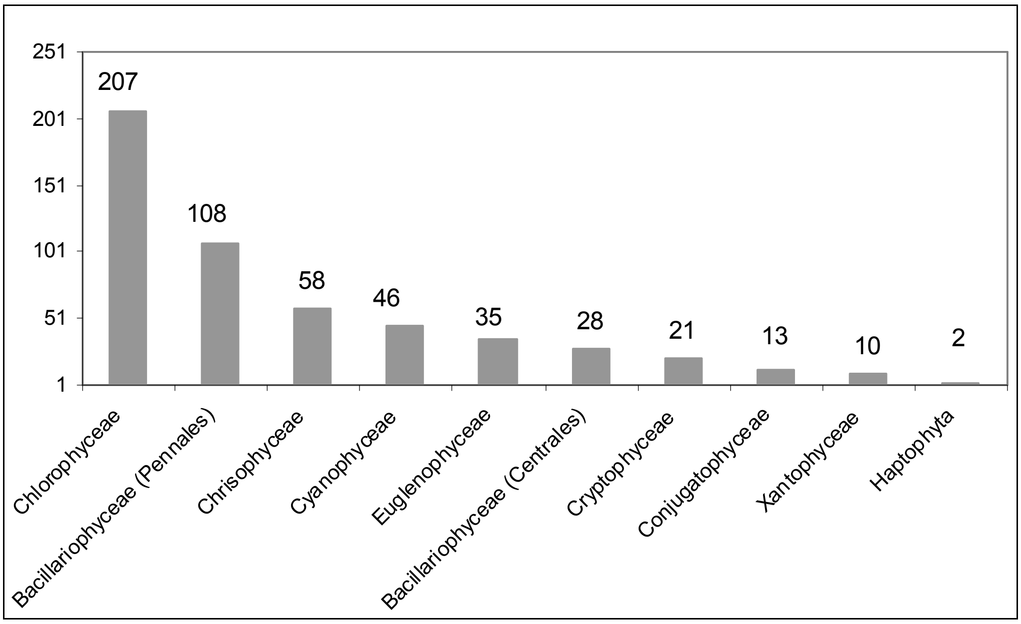

Figure 1. *The main phytoplankton groups and their species number between 1979 and 2002*

The occurence and specimen number of the species are far from even. Only a scarce number of species occur throughout a year. During the 24 years, only a small percent could be considered constantly present, however, coloring species (the ones that can be found 1 or 2 times a year or not even yearly) were relatively rare. The ratio of species that occur in maximum 20% of the samples was large, 89% of the identified species belong here (*Fig 2*). There is large distinction between the first and the other groups (where species occur at least in 20% of the samples), whilst there is slighter difference among the groups with more common occurrences (at least 60%, 80% or even more frequent). Less than 1% of the taxa were found in more than 80% of the samples.

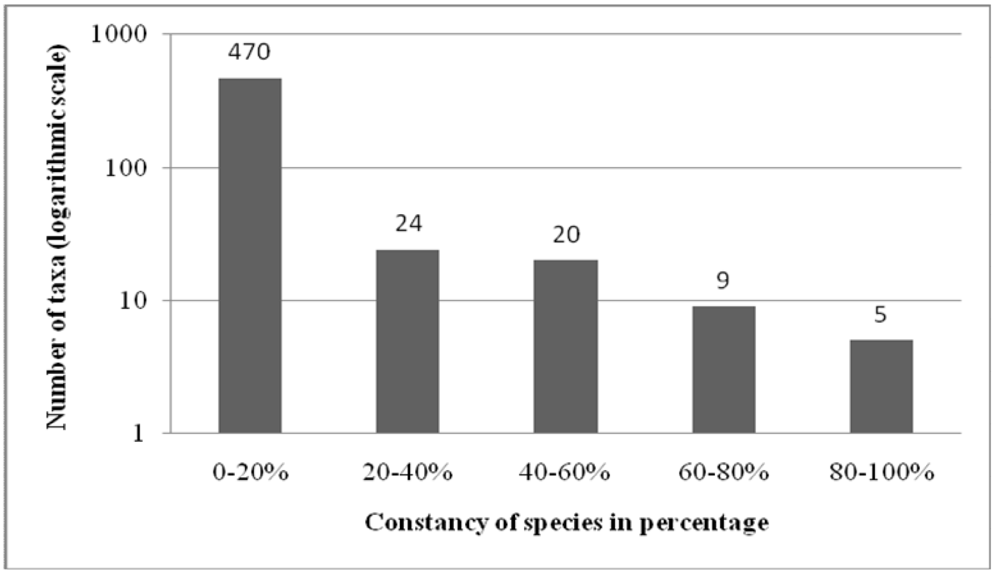

Figure 2. *Appearance likelihood in percentage of the taxa collected from the Danube at Göd during 1979-2002, sorted into 5 groups. The idea was taken from Du Rietz (1921)*

For the long-term analysis the phytoplankton density was measured in ind ml^{-1} and mg l^{-1} biomass means (*Fig. 3*). Between each year a considerable difference is present, big jumps in density between years are rotating as well, as short and long tendencies. While between 1960 and 1980 the phytoplankton numbers in the Danube were nearly duplicating, this trend seems to be reversing to the millennia. During the beginning of the 80s there was a period (1981-1983) where 30000 ind ml^{-1} of average abundance and 17-19 mg l^{-1} of biomass occurred. This period was followed by a recession in phytoplankton numbers, which lasted till 1987. The 90s could be characterized with 2 periods of high densities of phytoplankton, although this time the maximum of the average abundance was 20000 ind. ml^{-1} and the biomass 11-14 mg l^{-1}. In the very last years of this study the abundance diminished to 10000 ind. ml^{-1} and the biomass 4-5 mg l^{-1}. The trendlines indicate a clear decrease in density and in the magnitude of the phytoplankton biomass.

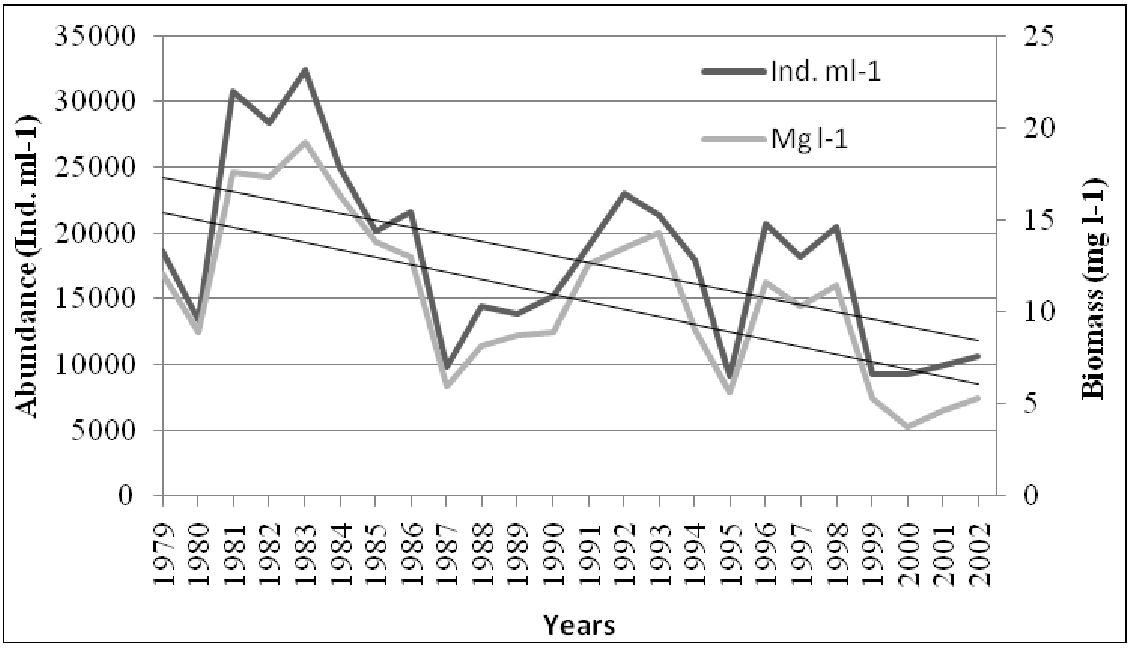

Figure 3. *Phytoplankton abundance (ind. ml^{-1}) and biomass (mg L^{-1}) means in each year at Göd and the trendlines*

The change of phytoplankton abundance can be observed in more detail on *Fig. 4*. Early years (1981-1985) show late winter blooms and summer periods with intensive algal growth. The highest numbers in phytoplankton were found during these blooms. Such density in summer can be found only in these early years. After 1985, for nearly 5 years, late winter blooms of the community disappeared. In the latter years (1991-1998) only late winter and early spring blooms were found in the samples and the other ones disappeared.

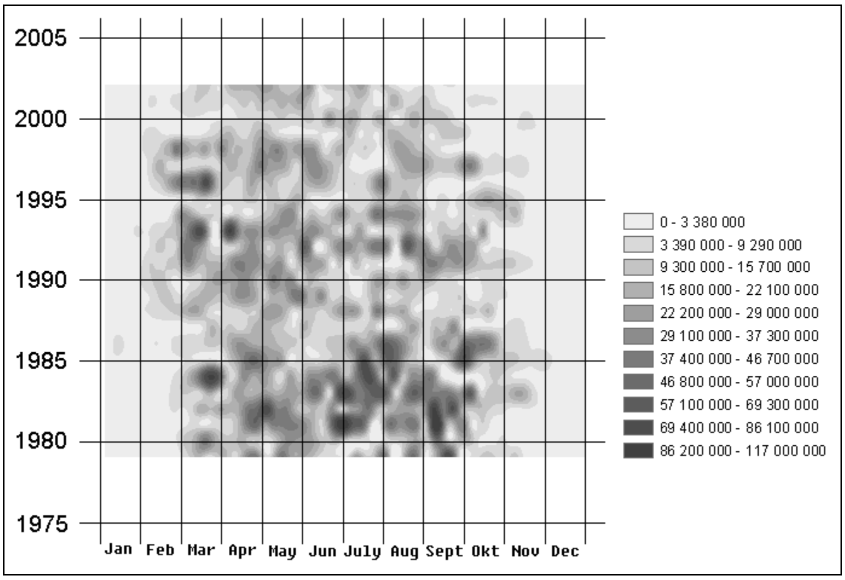

Figure 4. *Quantitative fluctuations of the phytoplankton of the Danube River between 1979 and 2002 with three-dimensional imagery. The x axis represents months, the y axis years. On the right side of the graph the shadows indicate the abundances in ind.* l^{-1}

Regarding the *Stephanodiscus* spp. group (consist of those 9-12 near related species from the *Stephanodiscus, Cyclotella, Thalassiosira* és *Cyclostephanos* genus, that cannot be identified with light microscope during the Utermöhl' counting) more accurate observation can be made while analyzing its 3D graph (*Fig. 5*). Compared to the previous graph, here the absence of summer blooms are more emphatic after 1985. In the 90s the *Stephanodiscus* spp. peaks are only detectable in the early spring periods. The seasonal dynamic of this group is not steady, for some reason the former conspicuous Centrales peaks have been missing the later years.

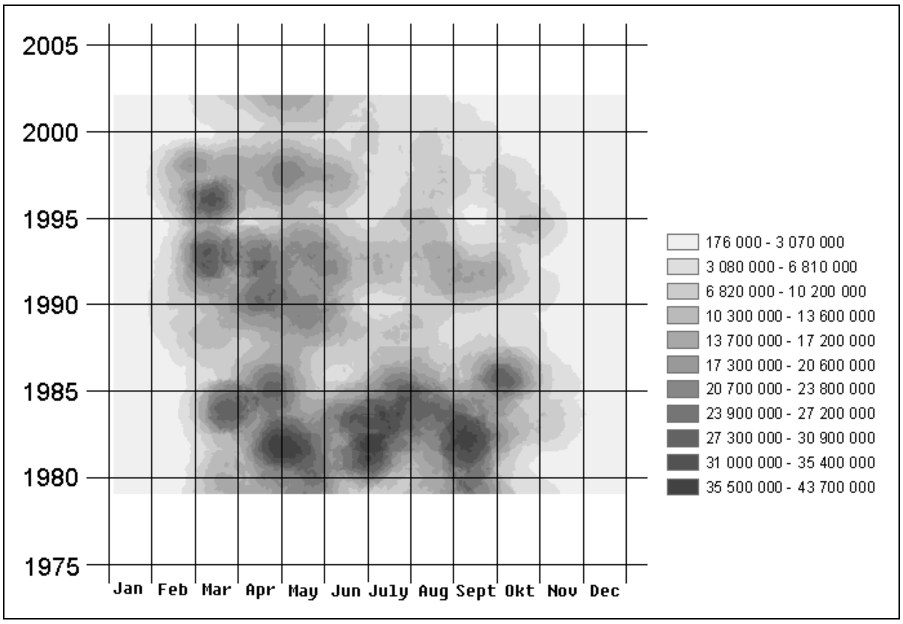

Figure 5. *The Stephanodiscus spp. abundance between 1979 and 2002 with 3D-imagery. The x axis represents months, the y axis years. On the right side of the graph the shadows indicate the abundances in ind.* l^{-1}

Diversity indices

To measure the biological diversity of a community, using diversity indices of different kind is a very useful tool, each providing slightly different information about the community structure in question and about the changes in the background. Diversity indices are formed from two components of empirical approach, the evenness and the richness component. Since an accurate definition for biodiversity cannot be given, we examined many diversity indices (Shannon H, Simpson 1-D, Evenness e^H/S, Menhinick, Margalef, Equitability J, Fisher alpha, Berger-Parker). Comparing these diversity indices we have found they all show similar seasonal dynamics, so in this paper we only present the Shannon index in detail.

The phytoplankton community diversity has ascending and descending periods during the years (*Fig. 6*). In the very early years diversity is high, which drops down and a new peak was shown only 10 years later in the early 90s. Then diversity started to decrease again, which lasted till 1998. In the last years of the study we measured another rise with characteristically higher minimum values in winter. Compared to these last years, earlier minimum and maximum values were following a periodic pattern, in winter with low, in summer with high diversity values, respectively.

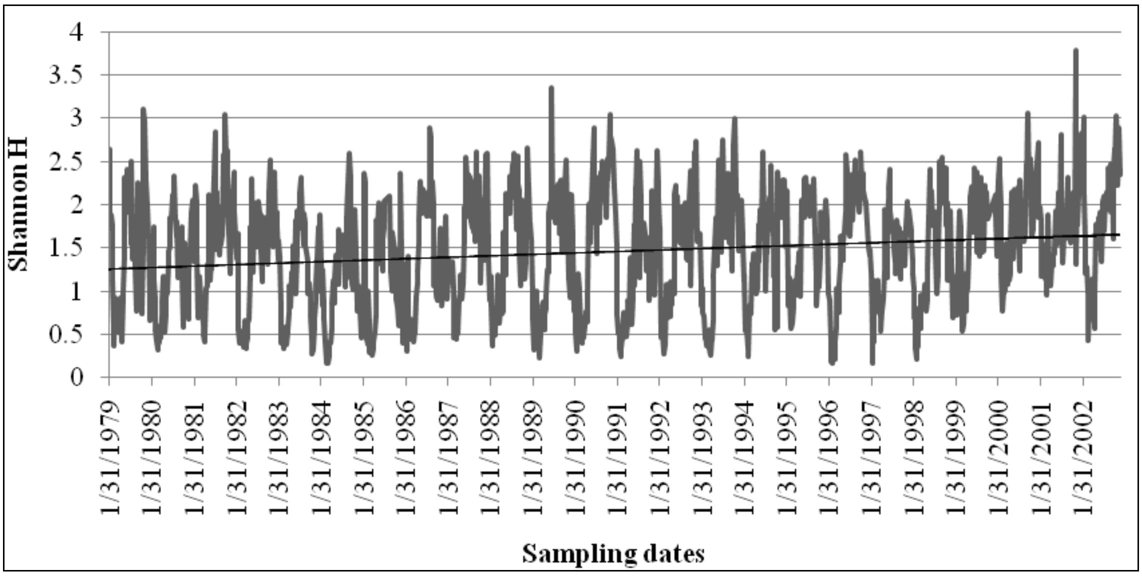

Figure 6. *Shannon diversity of phytoplankton of Danube from 1979 till 2002 and the Trendline*

To compare each year, the 3D-imagery of the Shannon diversity is forcibly descriptive (*Fig. 7*). Late winter and early spring periods have the lowest diversity indices. Only a few species are to be responsible for this. Also these low diversity periods appear at a slightly different time during the years. In the early years of 1979-1984 periods started at the end of February or at the first days of March, however, from 1985 low diversity periods started to appear a little earlier. Between 1988 and 1999 the diversity was low throughout February. We have observed higher diversity values in the summer periods of the latter years. The highest peaks of diversity can be found in late November and December. This shows the importance of the continuous sampling during winter. In December 1991 no sample was collected, that is why the diversity is zero at that time.

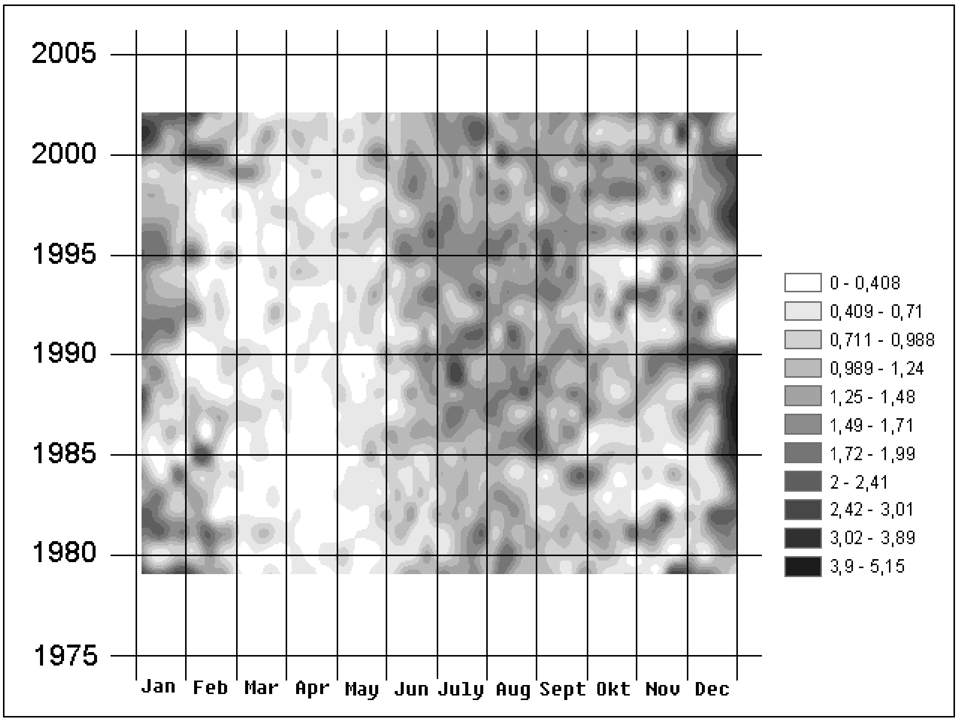

Figure 7. *The Shannon diversity of the phytoplankton of the Danube between 1979 and 2002 with 3D-imagery. The x axis represents months, the y axis years. On the right side of the graph the shadows indicate the diversity values*

Each year is easily characterized by their diversity indices means (*Fig. 8*). Comparing *Fig. 8* with *Figure 3*, a different gradient, but similar periodicity can be seen. In 1983 the Shannon index was especially low. In *Fig. 8*, three distinct terms are noticeable. In the first years of the study a strongly wavering diversity were measured, which was followed by a low diversity period (1983-1985). From 1985 this was switched by a transitory period that lasted till 1998. From that year, a new, strengthening term is observable with the highest average values of 1,8-1,9 in the entire study. In these last years, as it could have been forecasted from the lesser abundance and biomass values, few abundant Centrales species diminished from the phytoplankton community so other rare species gained more emphasis. All in all, the highly fluctuating years were followed by a transient period with growing diversity, followed by unambiguous diversification.

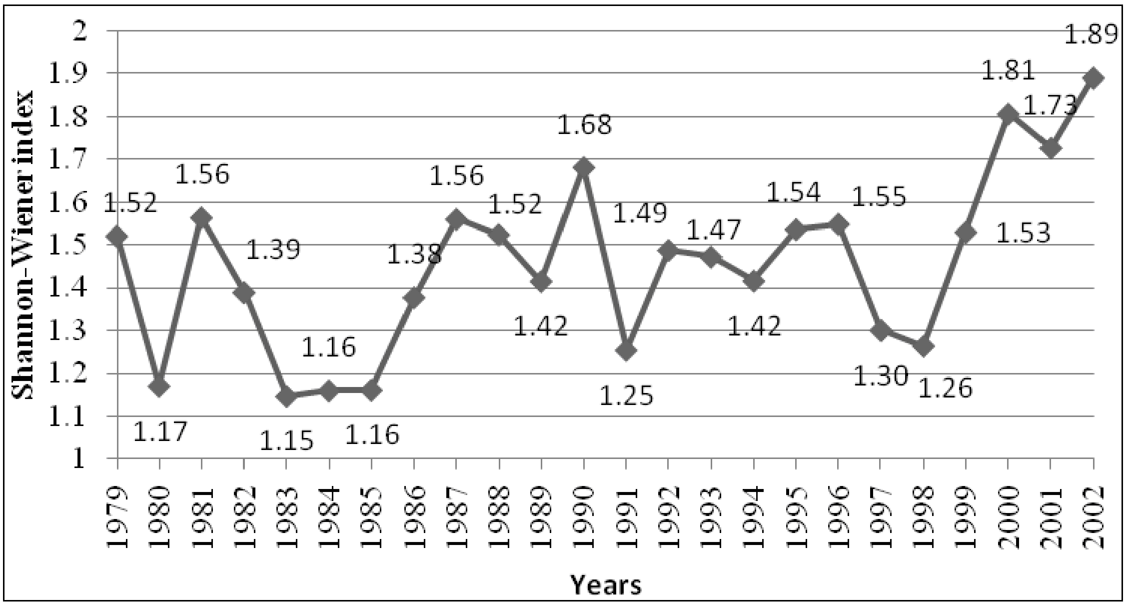

Figure 8. *Means of Shannon diversity in each year estimated from the phytoplankton samples in the examined period of 1979-2002*

Strong relation was found between values of abundance/biomass and values of diversity. Most years show low diversity in the late winter periods because of the winter blooms of algae (for example 1984, 1993, 1996), however, there were certain years where the late winter blooms were missing but diversity remained low (1987, 1988, 1995). There were a few rare occasions where the diversity is unnaturally high in the winter periods. Such years included 1979, 2000 and 2002. The summer / early autumn periods were similarly diverse. 1983 and 1992 have characteristically high diversity and high biomass values. In 1985 and 1989-1990, the summer peaks were either very short or absent, whereas the biomass was high compared to the other years. In this paper we only show 2 representative years (*Fig. 9*).

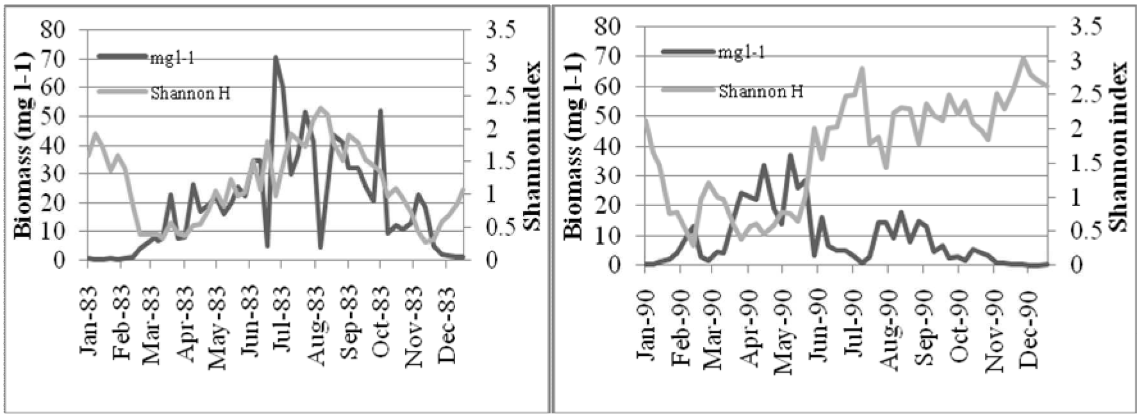

Figure 9. *Two years' (1983, 1990 biomass and Shannon diversity values. Both years had high diversity in winter. In 1983 after the summer bloom, species disappeared and the diversity fell, while in 1990 the diversity increased significantly*

We have investigated every year's most dominant species. Obviously we haven't found the same species every year, since with the diversity increase, we expected that some species would spread, some would disappear. We have found that each year, the 20 most abundant taxa provides 90-95% of the whole biomass, except for short winter periods. Observing each year, between 1979 and 2002, 63 taxa ranked in the Table of ranks (*Table 1*). Each taxa is coded with a number (according to their alphabetic order) and their ranks change during the years. For an easier understanding some cells were shadowed differently. Following the species rankings through the years is much easier. Every year the *Stephanodiscus* spp. (Code 481) ranked in the first place. In the second place mostly *Skeletonema potamos* (Code 467) was found, sometimes replaced by *Stephanodiscus neoastraea* (in 1979, code 466), *Stephanodiscus binderanus* (in 1980, code 480) and *Pandorina morum* (in 1985, code 393), *Coelastrum microporum* (in 1993, code 129). They are rather abundant in the first part of the examined period: *Oocistis borgei* (code 374), *Skeletonema neoastraea* (code 466), *Stephanodiscus hantzschii* (code 449), *Trachelomonas volvocina* (code 503) and *Chlamydomonas sp* (code 86). There are rather abundant in the second part of the examined period: *Fragilaria ulna* (code 224), *Aulacoseira subarctica* (code 12) and *Planktothrix aghardii* (code 419).

Table 1. *Table of ranks. Every taxa is coded with numbers for a better overview. Each year (columns) consist of the 20 most abundant taxa's code. The names behind the codes can be found in the Appendix*

	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
1	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481	481
2	466	**480**	467	467	467	467	393	467	467	467	467	467	467	467	129	467	467	467	467	467	467	467	467	467
3	467	466	**480**	449	129	129	467	129	129	129	129	129	129	129	467	129	129	480	129	129	129	**480**	**480**	130
4	129	467	129	**480**	130	393	129	393	250	393	250	130	**419**	**12**	393	**419**	46	129	**419**	393	43	129	43	129
5	43	43	320	129	449	363	130	130	43	250	43	12	**480**	393	**12**	130	393	**12**	**480**	43	**419**	**419**	129	250
6	86	449	130	130	**480**	130	43	167	130	130	**419**	224	393	**419**	**419**	46	130	393	250	349	393	393	224	**419**
7	363	393	518	503	167	167	449	363	363	**419**	320	320	403	130	130	403	224	130	224	130	**12**	**12**	320	58
8	320	86	503	393	320	224	167	250	167	320	130	167	43	224	43	393	250	46	43	403	130	130	130	320
9	148	129	363	363	393	86	363	58	58	**480**	363	363	**12**	43	224	224	**419**	349	**12**	**419**	487	58	**419**	224
10	46	224	186	320	374	43	20	**480**	320	43	403	58	224	400	320	320	43	224	320	58	58	167	58	**480**
11	176	130	167	86	46	320	58	20	393	21	258	250	130	320	487	108	**480**	**419**	130	**12**	353	46	363	108
12	58	320	86	43	186	224	374	374	86	167	58	21	216	363	46	216	58	487	46	224	250	403	85	363
13	20	363	22	167	86	366	86	86	224	363	167	85	58	167	400	58	108	403	58	167	363	108	108	43
14	91	487	20	374	363	181	22	401	374	22	85	374	363	20	20	250	320	58	349	363	224	320	167	167
15	167	503	46	58	522	186	88	65	22	85	20	342	401	216	363	363	209	43	167	108	320	25	251	46
16	304	58	58	366	43	58	366	366	176	20	21	393	46	46	58	43	363	108	108	250	167	490	**12**	393
17	22	173	374	224	58	20	320	46	366	374	22	176	20	58	216	167	487	468	363	487	85	363	250	487
18	503	20	181	46	143	374	445	88	224	58	86	43	167	21	353	20	216	353	400	85	108	85	487	85
19	395	374	91	486	22	176	108	186	85	176	181	20	108	401	22	21	353	400	85	374	22	65	46	374
20	487	181	366	186	445	151	145	108	216	487	176	108	250	374	172	316	12	342	374	214	374	487	353	22

Species abundance models

It can be proved from the models that in the case of the smoothest relations and the most abundant species, the simultaneous broken stick model approximates the abundance-distribution the best. At the same time this means the upper barrier of the different models. With the decrease of the resources and the abundance of the species the models can be ranked after each other as follows: beta-distribution, lognormal distribution, and geometrical distribution.

In *Figure 11* the graphs of abundance distribution, lognormal distribution model and beta-distribution model can be seen based on the total harnessing between 1979 and 2002. It can be noticed that at the beginning (in case of species with large abundance) the graph suits the logarithmic model, while with species of smaller entity numbers it follows beta-distribution.

The reason can be that species with smaller entity numbers are less rivals of each other in the consuming of remaining resources that were left by the species with larger abundance. The other possible reason is that the species with smaller entity number distribute more smoothly in time (seasonally), while the abundance of species with large entity number is season-dependent.

The comparison of the gradation of phytoplankton, beta-distribution and lognormal distribution (*Fig. 10*):

Beta: In case of α= 2,3 and β=0,0001 the correlation of the gradation and beta-distribution is 0,997643.

Lognormal: In case of σ= 1,8 and μ=0,00001 the correlation of the gradation and lognormal distribution is 0,885658775.

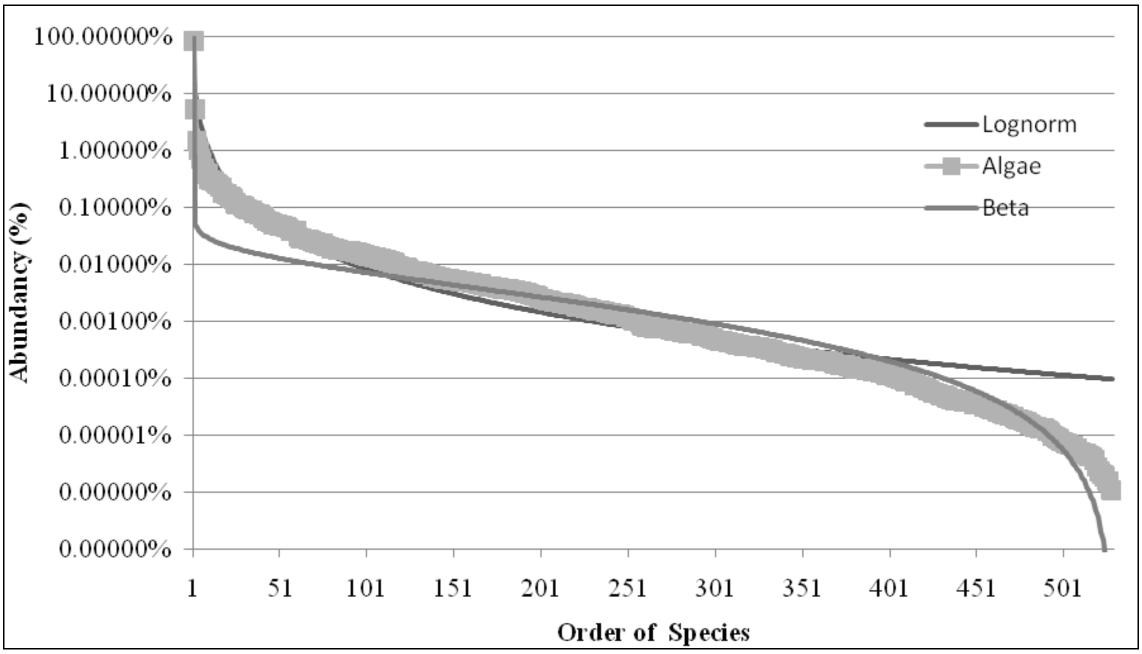

Figure 10. *The comparison of the gradation of phytoplankton, beta-distribution and lognormal distribution*

Similarity patterns of the phytoplankton assemblage

In the first investigation the datamatrix consist of years as sample units and the abundance of the taxa as variables. Our hypothesis is that the patterns were determined by the dominant species, the ones abundant every year, so we tried to interpret the results of the multivariate methods with the columns of the ranking table (*Table 1*). Ordinations were done both with ind. ml^{-1} and mg l^{-1} data; however, these ordinations did not differ significantly. We have considered the biomass data more relevant, according to the general algological literature.

On the grounds of the NMDS and Cluster Analysis we can set the next statements about *Fig 11 and 12*:

The groups cannot be clearly separated in the ordination plot. In the bigger group that is the centre of ordination plot we can sparely find some from the 1980s and 90s. Year 1979 also belongs to this group which is a little bit different from other years. In 1979, *Stephanodiscus spp*. did not have a peak, but *Stephanodiscus neoastraea* ranked in the second place with such high biomass that only *Skeletonema potamos* could exceed. In other years of the group *Stephanodiscus spp.* is characterised by less biomass but the biomass of the species has decreased as well.

In 1981-1982 the biomass of *Skeletonema potamos* escalated into a peak and ranked in the second place. At the same time the first *Stephanodiscus spp.* peak occurred (compared to 1980, their biomass doubled). On the dendogram, the year 1981 and 1982 is separated at a very early stage. In center of the ordination plane the late 1980s and early 1990s compose a group. In these years *Stephanodiscus spp.* have lower biomass and other species diminished as well. Aside from this, the year 1994 forms a separate group. In this year *Skeletonema potamos* has a salient peak.

The last group was formed from the years 1995, 1999, 1983, 1984, 1985 and the 2000s. Although in these years *Stephanodiscus* spp. and *Skeletonema potamos* occupied the first 2 places, their biomass were only half and seventh of the previous years'. In these years every dominant species' biomass was smaller. 1985 and 1999 belong in this group; however, their similarity could not be interpreted by the ranking of the dominant species, except for the increase of *Pandorina morum* in 1985.

No other significant separation could be interpreted by the table of ranks

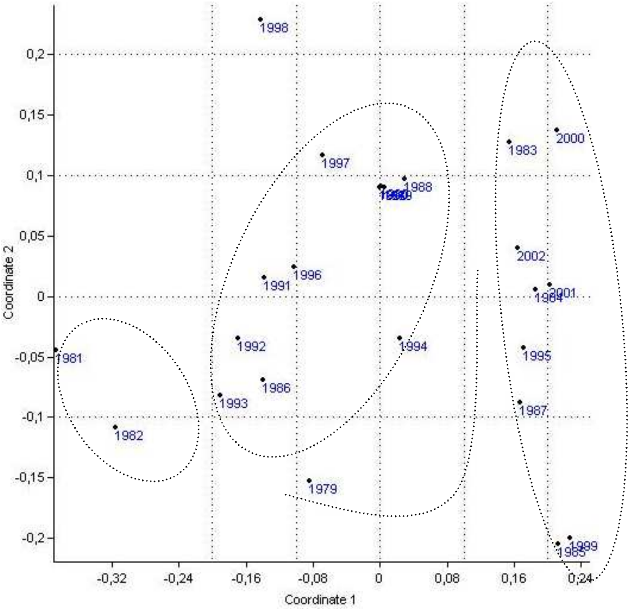

Figure 11. *Ordination of the studied years with NMDS method for every taxa (Euclidean distance)*

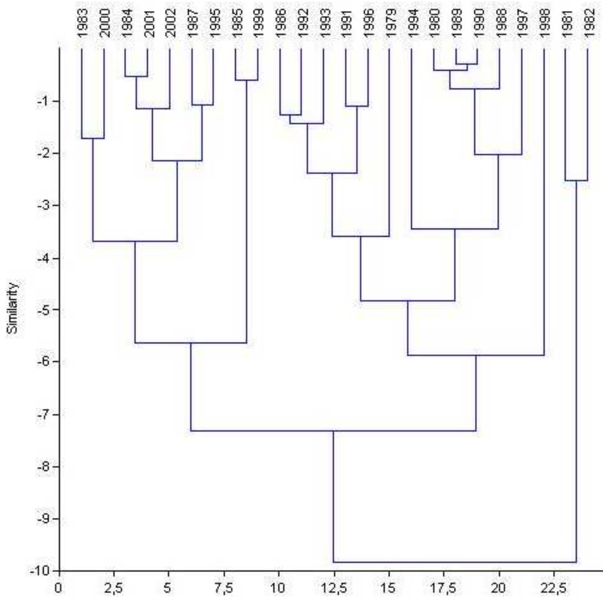

Figure 12. *The dendogram for every taxa (Euclidean distance)*

Similarity patterns based on logarithmic transformation of the data matrix

We assumed that the most abundant species have influenced the previous analysis. A logarithmic transformation of the data tempers the (order of) magnitude in difference and decreases the emphasis of the dominant species. The standard method is to add 1 to every value, however, we only added 1 to the 0 values (absence of the phytoplankton), since the standard method would have distort the biomass data (from asymmetric to the right distribution to an asymmetric to the left distribution).

A temporal trend can be observed in the groups (*Fig. 13* and *14*). On the one hand, long-term changes were clearly indicated: the first (1979-1990) and the last (1991-2002) part of the examined period can be clearly separated. On the other hand, each year is followed by the next one in the ordination plot till the end of the 1980s, where we suspect a great change in the phytoplankton composition.

The separation of these periods cannot be explained with the ranking or the abundance. The years 1989, 1990 and 2002 have the highest species number. Each year had over 220-230 species. After the logarithmic transformation these new species got a strong emphasis. Surveying each year's taxonomic composition we found, that for example in 1990, there were 70 species that disappeared to 1991 and there were only 33 new species in 1991.

The 1990s and the 2000s have separated clearly as individual groups, but the temporal trend is harder to notice. 1995, as a year rich in species is separated clearly, same as the 2000s where the species numbers were also high.

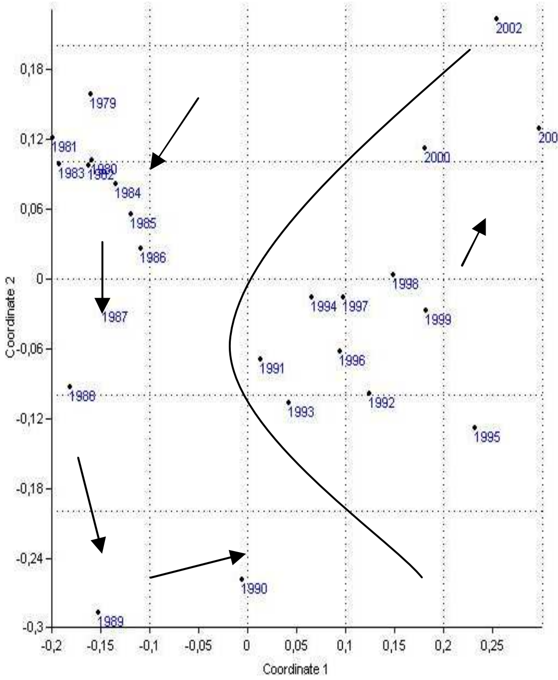

Figure 13. *After logarithmic transformation of the data, the NMDS ordination of the studied years with Euclidean distance*

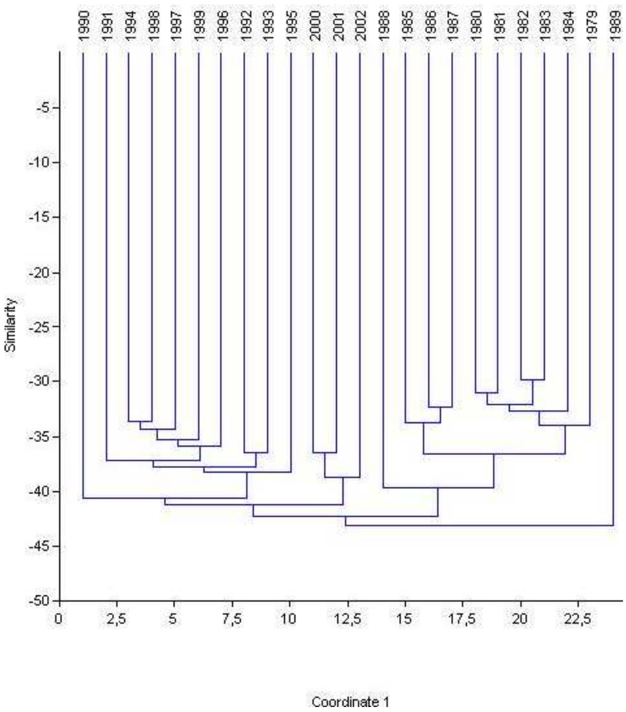

Figure 14. *After logarithmic transformation of the data, the dendogram of the studied years with Euclidean distance*

A seeded scale type, the binary variable

In most cases one variable can take infinite values. The strongest reduction of this is the binarization, which equals the species presence/absence characteristic. On this scale, we are only interested in what effect each species have on the whole community regardless of their abundance (may it by ind ml^{-1} or mg l^{-1}). In this case (*Fig. 15* and *16*) we observed an even stronger "arch effect" and continuous transition during the years.

The earlier (1979-1990) and latter (1991-2002) years behaved like in the results of the logarithmic transformation. The contingency of the vast number of coloring species is clearly noticeable, this can be observed on the Cluster Analysis, the years separate very early. A temporal trend is clearly recognizable on the ordination plot like in the results of logarithmic transformation.

A clear separation of the years 1988-1990 can be seen, which was attributed to the high species number (only here has the Hierarchical Clustering significant change).

The highest diversity characterised the last years of the study. It can be explained by the decrease of phytoplankton biomass that is due to the decrease in abundance of dominant species.

On the whole we can assess that when no quantitative effect influences the assays, the main organizing order is the number of rare species each year. The pattern of occurrence looks to be a continuously shifting gradient in the ordination plot. The cluster analysis support additional information: the phytoplankton in the Danube River, that is rich in coloring species, is very diverse each year.

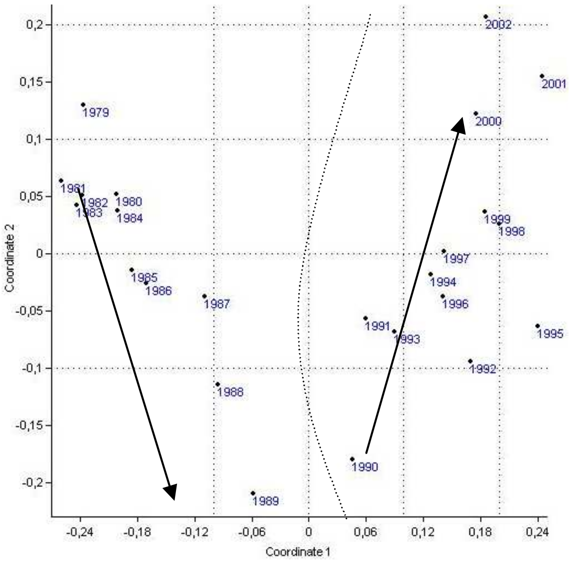

Figure 15. *NMDS ordination of the studied years with Euclidean distance from binary data*

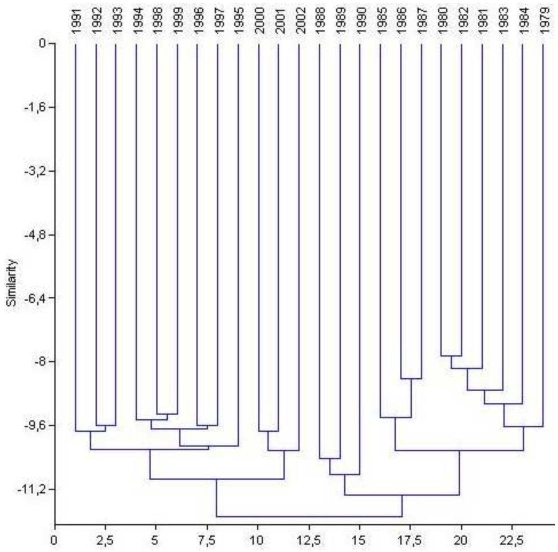

Figure 16. *Dendogram of the studied years with Euclidean distance from binary data*

Discussion

The potential trophic level of River Danube based on nutrient supply (P, N) is polytrophic in Hungary (Déri, 1991). Therefore in suitable circumstances it can become polytrophic with dense phytoplankton. The actual trophic level changes during the year according to phytoplankton biomass, chlorophyll-a concentration, primary production (Kiss, 1994; Vörös et al., 2000). The river is oligotrophic in December-January, it can become mesotrophic by the end of February in low water periods. From spring to autumn the river is oligo-mesotrophic during the floods and is eutrophic, polytrophic during the low water periods. It is remarkable that certain years the river reaches eu-polytrophic level by March, too (Kiss, 2000).

An important ecological factor controlling the density of phytoplankton is the water transparency, influenced first of all by floods. Light climate of rivers like the River Danube is determined primarily by the quality and quantity of suspended matter and the thickness of the euphotic zone. The suspended matter content of the River Danube is low, transparency is high in low water period and vice versa. The depth of the euphotic zone is 250-300 cm (80-90 % of water column) in low water period and 100-130 cm (25-30 %) during the floods. The effect of dilution, change in current is much less controlling the abundance (Kiss, 1994).

According to recent research we question our previous statement of a river being perfectly homogeneous. We can presume the competitive exclusion theory (Hardin, 1960) is not effective, species from brooks to water reservoirs survive in the Danube for a very long time. The 90% rate of the coloring species supports this hypothesis. In a reservoir detached for years from the mainstream, a still water community is to be developed, which is usually limited by nutriments. From such reservoirs more than hundred were built in the 1960s and 80s along the Danube and it tributaries.

The inner dynamic fluctuations of the plankton community could have a stabilizing effect on the large diversity of the community structure (Huisman and Weissing, 1999). Stress and disturbance events affecting the community (Jacobsen and Simonsen, 1993; Barbiero et al., 1996) could result in the community's incompetence of reaching equilibrium state with little species numbers, as the mathematical models predict.

We have appointed that diversity changes in the late winter periods have been starting a little earlier from 1985. The increasing magnitude of flushes and the slow increase in water temperature, which were measured during the reported period also suggest, extreme climatic events (IPCC, 2007; Kiss, 2000) have great effect (Sipkay et al. 2009, 2010) on the phytoplankton. Many research studying global warming predicted earlier phytoplankton biomass maximums (Flanagan et al., 2003; Wiedner et al, 2007; Sipkay et al, 2009), especially in the winter periods (Thackerey et al, 2008). It is possible that the phytoplankton of the Danube after 2002 will favor this hypothesis.

The multivariate analyses have led us to different results. The patterns were determined by the dominant species based on the results of the first investigation. The reason we couldn’t separate the groups clearly might be the high representation of dominant species. In cases of logarithmic transformation and binarization of the datamatrix the temporal patterns could be unambiguously recognized. The temporal trend in the examined period (years following one another) can refer to the possibility of the existence of gradually changing environmental factor. Assumable the changes in the degree of nutrient excess could cause this. The economic and environmental consequences of change of regime, that was significant historical event in Hungary, could lead to separation of the two period. The breakdown of the Socialist large-scale industry and the development of sewerage could lead to the decrease of nutrient load in the Danube. According to the statistics the nutrient load has decreased by 40-50 % in Danube’s watershed (Schreiber et al. 2005, ICPDR 2005, Csathó et al. 2007).

This study supports the presumption that despite the food oversupply in the Danube, the community stand in the door-step of an era without large algal blooms. Future monitoring and modeling studies shall have an answer.

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APPENDIX

Code	Phytoplankton taxa (Danube)
3	*Aulacoseira italica var. subarctica (O. Müll.) Sim.*
12	*Aulacoseira subarctica (O.Müller) Haworth*
20	*Actinastrum hantzschii Lagerh.*
21	*Actinocyclus normanii (Greg.) Hust.*
22	*Acutudesmus acuminatus (Lagh.) Tsarenko*
25	*Amphora ovalis Kütz.*
43	*Asterionella formosa Hassal*
46	*Aulacoseira granulata var. angustissima (O.Müll.) Sim.*
58	*Cryptomonas ovata Ehrbg.*
65	*Closterium strigosum Bréb.*
85	*Chlamydomonas reinhardtii Dang.*
86	*Chlamydomonas sp.*
88	*Chlamydomonas sp. Nagy (oval)*
91	*Chlorella sp.*
108	*Chroomonas acuta Uterm.*
129	*Coelastrum microporum Näg.in A.Br.*
130	*Coelastrum sphaericum Näg.*
143	*Crucigeniella apiculata (Lemm.) Kom.*
145	*Cryptomonas erosa var. reflexa Marss.*
148	*Cyclotella meneghiniana Kütz.*
151	*Cymatopleura solea (Breb.) W.Smith*
167	*Dictyosphaerium pulchellum Wood*
172	*Dictyosphaerium tetrachotomum Printz*
173	*Diatoma vulgaris v. brevis Grunow*
176	*Desmodesmus communis (Hegew.) Hegew.*
186	*Dictyosphaerium ehrenbergianum Näg.*

209 Euglena texta (Duj.) Hübn.
214 Fragilaria teñera (W. Smith) Lange-Bertalot
215 Fragilaria ulna v. oxirincus (Kütz.) Lange-Bert.
216 Fragilaria ulna var.acus (Kütz.) Lange-Bert.
250 Gymnodinium sp.
251 Gymnodinium sp. kicsi, kerek
258 Hannaea arcus (Ehrgb.) Patrick
304 Monoraphidium contortum (Thur.) Kom.-Legn.
316 Melosira varians Agardh
320 Micractinium pusillum Fres.
342 Nitzschia palea (Kütz.) W.Smith
349 Nitzschia tryblionella Hantzsh
353 Navicula cryptocephala Kütz.
363 Nitzschia acicularis (Kütz.) W.M.Smith
366 Nitzschia sp.
374 Oocystis borgei Snow
393 Pandorina morum (O.F.Müller) Bory
395 Pennales sp.
400 Peridinium cinctum (Müller) Ehrbg.
401 Peridinium sp.
403 Peridiniopsis kevei Grigorszky
419 Planktothrix aghardii (Gom.) Anagn. et Kom.
445 Scenedesmus ecornis (Ehrbg.) Chod.
449 Stephanodiscus hantzschii Grun.
466 Stephanodiscus neoastraea Hĺkansson & Hickel
467 Skeletonema potamos (Weber) Hasle
468 Skeletonema subsalsum (Cleve-Euler) Bethge
480 Stephanodiscus binderanus (Kütz.) Krieger
481 Stephanodiscus spp.
486 Strombomonas fluviatilis (Lemm.) Deflandre
487 Surirella brebissonii Krammer Lange-Bert.
490 Surirella robusta Ehrenberg
503 Trachelomonas volvocina Ehrbg.
518 Thalassiosira weissflogii (Grunow) Fryxell & Hasle
522 Trachelomonas sp.

ECOLOGICAL ASSESSMENT AND PHENOTYPIC AND FITNESS VARIATION OF SINAI'S REMNANT POPULATIONS OF *MORINGA PEREGRINA*

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Abstract. *Moringa peregrina* which is one of the most valuable and economically important medicinal species in the Egyptian desert has become one of the most endangered trees due to unmanaged grazing and over-collection. The present study aims to provide ecological assessment of the species and to investigate whether progeny from the remnant fragmented populations show reduced fitness. Sixteen sites containing a total of 197 trees were sampled upon survey of Wadis in South Sinai, where vegetation parameters and associated species were recorded. Variation in edaphic factors, phenotypic traits, germination, and early life-history fitness were assessed and analyzed. The results indicated that *M. peregrina* has narrow distribution and grows on cliffs and at the base of hills (300 - 800 m a.s.l.) with very rugged topography. Almost all the reproductive trees grow on south facing slopes and crevices of metamorphic rocks. The study clearly showed that the studied populations have very low early stage fitness estimated as an index of maternally affected life-history characters. The study suggested that the deteriorated environmental circumstances have affected negatively the fitness of maternal plants in small populations and the performance of their offspring. It concluded that direct protection is urgently needed to stop further deterioration of the populations and to improve their number and size.

Keywords: *Sinai, Moringa peregrina, conservation, fitness, medicinal plants.*

Introduction

Egypt, due to explosive growth of its population, needs to conserve, reallocate, and sustainably use its natural biological resources. A special concern should be directed to underutilized, rare and endangered but economic species. In the last thirty years, the wild vegetation of South Sinai has been endangered which resulted in disappearance of palatable species, rarity of trees, and change in vegetation composition (Moustafa *et al.*, 2001). Abd El-Wahab *et al.* (2004) assessed the status, distribution, the causes of loss, and the specific threats facing conservation and sustainable use of medicinal plants in St. Catherine Protectorate. The study recommended that detailed and specific reproductive ecology, life-history characteristics, and population genetic diversity and structure studies are essentials to construct sound conservation and sustainable utilization plan for the threatened medicinal species.

Due to the destruction, deterioration and fragmentation of their habitats, many species (including *Moringa peregrina*) have recently been forced into small and isolated populations. *Moringa peregrina* which belongs to the monogeneric family Moringaceae, with just thirteen/fourteen species throughout the dry tropics of the world (Somali *et al.,* 1984), is one of the most economically important medicinal plant species in Egyptian desert. *Moringa* family consists of about ten Xerophytic species (FAO, 1988; Al Kahtani and Abou-Arab, 1993), distributed from tropical Africa to the East

Indies. Although it is a rare species, *M. peregrina* has a wide geographic range, growing from the Dead Sea area sporadically along the Red Sea to northern Somalia and around the Arabian Peninsula to the mouth of the Arabian (Persian) Gulf. In Egypt, it is recorded from the eastern desert, Red sea coast, Elba massive, and the Sinai Mountains (Täckholm, 1974; Boulos, 1999). It is recorded in South Sinai in small limited areas at W. Feiran, W. Me'ar, and W. Zaghra and locally called "Yassar". It grows in crevices and rocky slopes of mountains. *Capparis cartilaginea*, *Reseda decursiva*, and *Hamada elegans* are the main associated species. *M. peregrina* is adapted to a wide range of soil types but it does best in well-drained loam to clayeyloam (Abd El Wahab, 1995). It does not withstand prolonged water logging. It is observed to prefer a neutral to slightly acidic soil reaction, but it has recently been introduced with temperature ranges from 26 to 40 ºC and annual rainfall at least at least 500 mm.

Moringa peregrina could soon become one of the world's most valuable plants, at least in humanitarian terms. Its seeds have different economic and medicinal importance due to its unique composition of oil (42-54%), fatty acids (14.7 % saturated, and 84.7 unsaturated), proteins (23%), fiber (3.6 %), and carbohydrates (15.3 %) in its kernel that contains 1.8% moisture, and 2.5% ash, (Somali *et al.*, 1984). They are the source of oil used by Egyptians since the old and middle kingdoms (3000-2000 BC) and currently are used in folk medicine "Al-Yassar" (Migahid, 1978; Abd El-Wahab *et al.*, 2004). The extracted oil is composed of 14.7 % saturated and 84.7 % unsaturated fatty acids including Palmitic (9.3 %), Palmitoleic (2.4 %), Stearic (3.5 %), Oleic (78.0 %), Linoleic (0.6 %), Araachidic (1.8 %), and Behenic (2.6 %) (Somali *et al.*, 1984). Refined oil has a yellowish color, a sweet taste, is non-sticking, and is odorless. As a result, it is highly valued for preparing cosmetics, cooking, and lubricating purposes. It does not turn rancid, is excellent on salads, can be used for soap making, and burns without smoke (Price, 2000). It has a reputation as watch oil (Verma *et al.*, 1976). The seedcake left after oil extraction can be used as soil fertilizer or in the treatment of contaminated water (the usual case in most local Bedouin communities). It could be used (as well as suspensions of ground seed) as primary coagulants to remove a high proportion of the suspended bacteria (Von Maydell, 1986).

According to Duke (1983), *Moringa* leaves can be described as "phytoactive". They are a source of vitamin A and, when raw, vitamin C. They are a good source of B vitamins and among the best plant sources of minerals (Price, 2000). The calcium content is very high for a plant while phosphorous is low. Iron content is high. It is an excellent source of protein and a very low source of fat and carbohydrates. The leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine, which are often in short supply in the Bedouin diet. The nutritional value is even higher for its pods. Both leaves and pods supply several amino acids including arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine (e.g. Makkar and Becker, 1996).

Moringa peregrina is an extremely fast growing small tree. Perhaps the fastest-growing of all trees, it commonly reaches about three meters in height just 10 months after the seed is planted (Abd El-Wahab, 1995). Wildly, it is 5-15 m high, with grayish-green bark, long leaves, and bisexual yellowish white to pink, showy, fragrant flowers (Täckholm, 1974; Boulos, 1999). The flowers are good for beekeepers as they provide nectar. *M. peregrina* wood is very soft, it makes acceptable firewood which is a basic requirement in traditional Bedouin life in the Sinai.

Due to the over-stressing demand and use of *Moringa peregrina*, there are increasing threats facing its populations in wild. It is subjected to severe over-harvesting, overgrazing, over-cutting, and uprooting (for fuel and medicinal uses) as well as disturbance through unmanaged human activities. These activities have caused *M. peregrina* to become extremely rare in Egypt. The overall result has been the extirpation of whole populations and a reduction in size of the remaining populations. There has also undoubtedly been an erosion of genetic diversity. A loss of genetic variation may reduce the ability of local populations to adapt to changes in the environment and the potential for evolutionary change (Frankel and Soulé, 1981; Beardmore, 1983). The continued over-exploitation of natural populations threatens this species with local extinction unless these practices are stopped and natural populations are restored. In addition, since this species is a promising candidate for cultivation, the loss of genetic variation that accompanies the reduction of population sizes could jeopardize selection for increased productivity. Due to a shortage of native *M. peregrina*, Egyptian medicinal plant traders have to import pods of other *Moringa* species to cover the demand for *Moringa* products.

The present study aimed to provide ecological assessment of the species which will greatly assist the design of rehabilitation as a part of *in situ* and *ex situ* conservation program and to investigate whether progeny from the remnant and fragmented small populations of *Moringa peregrina* show reduced fitness.

Materials and methods

Study area

The study was carried out in St. Catherine Protectorate, which covers 4300 km^2 and lies in the middle of the triangular mass of mountains of the southern part of the Sinai Peninsula that is bounded to the east and the west by Aqaba and Suez Gulf, respectively. This triangular mass of mountains, with its apex at Ras Mohammed to the south, is 7500 Km^2 in surface area and formed of igneous and metamorphic rocks, chiefly granites (Said, 1990). This mass of mountains is intensively rugged and dissected by a complicated system of deep Wadis, some of which reach a considerable length (e.g. Wadi Feiran, and Wadi Zaghra) and some are shorter, narrow and steeper, and represent tributaries of the main Wadis (e.g. Wadi El-Arbae'en) (Said, 1990; Moustafa and Klopatek, 1995).

Moringa peregrina populations are recorded in the Wadi Feiran, Wadi Me'ar, and Wadi Zaghra basins where it grows in crevices and on rocky slopes of metamorphic mountains. Wadi Feiran represents one of the longest Wadis in Southern Sinai. It is bounded by igneous and metamorphic mountains with different varieties of dykes. Its main tributaries include Wadi El-Sheikh, W. Solaf, W. El-Akhdar, W. Nesrin, W. Tarr, W. Mekatab, and W. Alliat. The vegetation cover throughout the Wadi ranges between 5 and 10%. The Wadi basin supports vegetation consists of about 40 plant species and is dominated mainly by *Acacia tortilis* subsp. *raddiana*. The species that associate *A. tortilis* subsp. *raddiana* associations include *Achillea fragrantissima*, *Anabasis articulata, Artemisia judaica*, *Capparis sinaica*, *Echinops glaberrimus*, *Fagonia mollis, Fagonia arabica, Launaea spinosa*, *Lycium shawii*, *Mentha longifolia*, and *Retama raetam* (Zaghloul *et al.*, 2008).

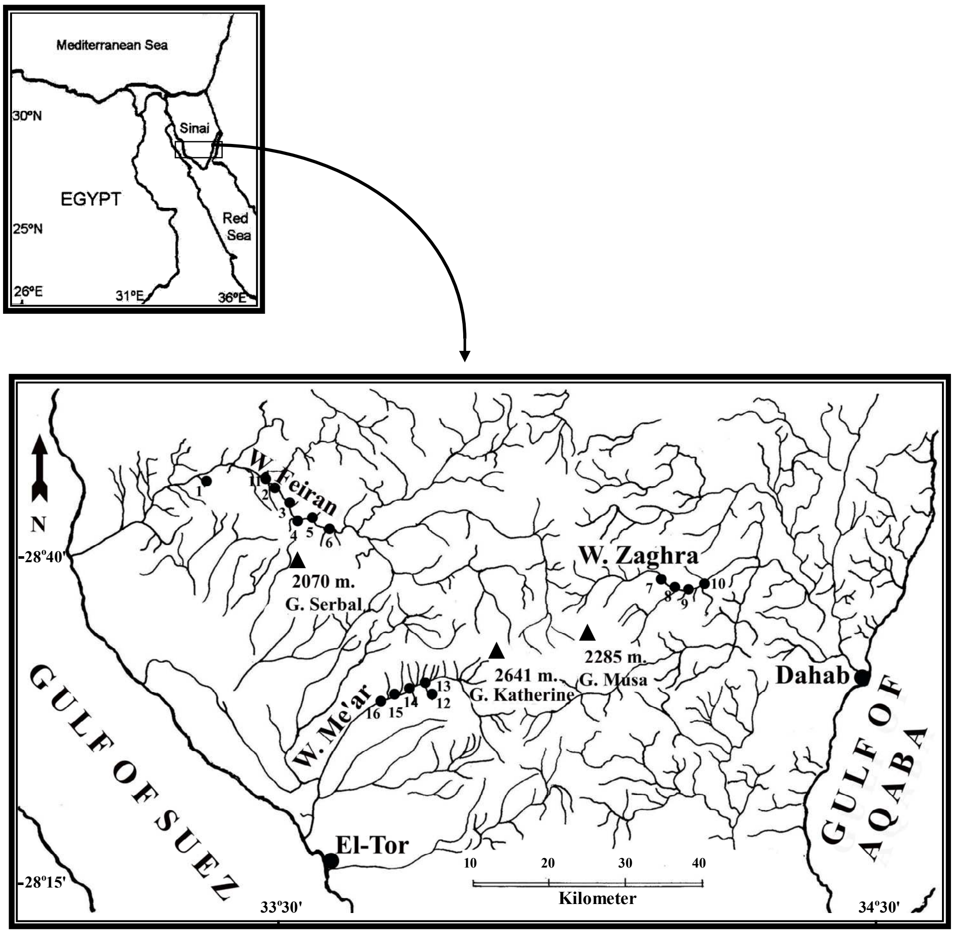

Figure 1. *Location map of the studied Moringa peregrina populations in Southern Sinai*

Wadi Me'ir is a rocky plain with more than 80% of the surface been covered with cobbles and stones, and about 20% with fine sand. The Wadi-bed is dissected with channels and deltas due to the water erosion. Generally, the total plant cover ranges between 1-5% allover the Wadi and 20-30% at some tributaries at the middle of the Wadi (Moustafa *et al.*, 1998). The following associations are recognized at the Wadi (Moustafa *et al.*, 1998): *Haloxylon salicornicum - Acacia tortilis* subsp. *raddiana*, *Haloxylon salicornicum - Zygophyllum coccineum*, *Acacia tortilis*, *Cleome droserifolia*. The associated species include *Achillea fragrantissima*, *Artemisia judaica*, *Capparis sinaica*, *Chrozophora plicata*, *Cleome droserifolia*, *Fagonia mollis*, *Iphiona scabra*, *Ochradenus baccatus*, *Panicum turgidum*, *Pergularia tomentosa*, *Zilla spinosa*, and *Zygophyllum coccineum*. *Moringa peregrina* is found as associated species or as co-dominant with *Hyoscyamus muticus* and/or *Fagonia mollis*. Wadi Me'ir is characterized by 50 plant species including one endemic and 26 medicinal species including 11 threatened species (Moustafa *et al.*, 1998).

Wadi Zaghra supports vegetation with cover percentage ranges between 1-5% in Wadi-bed, and 5-10% at foothill (Abd El-Wahab *et al.*, 2004). It supports the following main associations; *Haloxylon salicornicum*, *Solenostemma arghel*, *Moringa peregrina*, *Artemisia judaica*, *Zygophyllum coccineum – Aerva javanica*, *Moringa peregrina – Acacia tortilis*, *Acacia tortilis*, and *Retama raetam*. The associated species are: *Acacia tortilis* at the foot hills, *Capparis spinosa* on cliffs and *Aerva javanica*, *Cleome* *droserifolia*, *Ochradenus baccatus*, *Senna italica*, and *Crotalaria aegyptiaca* in the main Wadi (Abd El-Wahab *et al.*, 2004).

Medicinal plants in Wadi Zaghra include nine threaten species, only one of them is endemic, *Origanum syriacum*. These medicinal species include *Artemisia judaica*, *Capparis spinosa*, *Citrullus colocynthis*, *Cleome droserifolia*, *Cucumis prophetarum*, *Hyoscyamus muticus*, *Moringa peregrina*, *Pulicaria arabica*, *Senna italica*, *Solenostemma arghel*, and *Zygophyllum coccineum*. The following medicinal plant species are dominant in Wadi Zaghra: *Artemisia judaica*, *Fagonia mollis*, *Haloxylon salicornicum*, *Iphiona scabra*, *Moringa peregrina*, *Solenostemma arghel*, *Zilla spinosa*, and *Zygophyllum coccineum* (Abd El-Wahab *et al.*, 2004).

Threats affecting *Moringa peregrina* in the aforementioned Wadis include over-collection mainly of seeds for trade and of fuel wood for cooking and heating. Breaking down of the tree branches as a result of over-grazing effect is obviously shown around the area.

Table 1. *Summary of the number of Moringa pergerina trees, range of cover, and height of M. pergerina at the sixteen sites selected at three different wadis in South Sinai*

Location	Population Number	Number of trees	Cover (m^2)**				Height (m)**			
			Min	Max	Mean	SD	Min	Max	Mean	SD
W. Feiran	1	4	1.26	11.78	6.74	2.87	0.4	5.0	2.95	1.09
	2	6	2.51	14.13	7.09	4.76	1.0	5.0	2.75	1.54
	3	40	1.88	8.64	5.28	2.22	0.8	4.5	2.84	1.17
	4	6	1.88	14.13	7.56	4.72	1.0	5.0	3.08	1.69
	5	8	1.88	26.69	13.68	8.65	1.0	18.0	8.31	5.9
	6	11	8.64	21.98	15.70	5.38	3.0	10.0	6.81	2.86
	11	1	14.92	14.92	14.92	-	6.0	6.0	6.0	-
Subtotal	***7***	***76***	***1.26***	***26.69***	***8.11***	***5.61***	***0.4.***	***18.00***	***3.83***	***2.56***
W. Zaghra	7	8	26.69	39.25	29.20	5.62	13.0	21.0	17.0	3.81
	8	21	3.93	34.54	20.34	7.24	3.5	20.0	9.83	4.61
	9	12	4.71	31.40	17.40	7.88	4.0	15.0	9.96	3.76
	10	1	29.83	29.83	29.83	-	12.0	12.0	12.0	-
Subtotal	***4***	***42***	***3.93***	***39.25***	***20.81***	***8.01***	***3.50***	***21.00***	***10.30***	***4.93***
W. Me'ar	12	17	3.14	13.35	7.76	3.53	2.5	7.0	4.76	1.39
	13	27	1.18	11.78	6.44	2.97	1.0	8.5	3.57	1.98
	14	15	1.57	8.64	4.81	2.37	1.0	5.5	2.77	1.4
	15	15	1.57	14.13	6.9	3.86	1.0	8.0	4.4	2.47
	16	5	1.57	5.5	2.83	1.81	1.0	3.0	1.5	0.87
Subtotal	***5***	***79***	***1.18***	***14.13***	***6.28***	***3.33***	***1.00***	***8.50***	***3.70***	***2.00***
Total	**16**	**197**	**1.18**	**39.25**	**9.86**	**7.69**	**0.40**	**21.00**	**5.09**	**4.05**

Note: * = $P \leq 0.05$, ** = $P \leq 0.02$, NS = non significant variation

Field survey and seed collection

Three Wadis known to support *M. peregrina* trees were surveyed and sixteen sites were sampled (*Figure 1* and *Table 1*): six sites at W. Fieran (seventy-six trees), four at W. Zaghra (forty-two trees), and six at W. Me'ar (seventy-nine trees). Associating species, human activities, and land use were recorded to assess the ecological status of the *M. peregrina* populations in selected Wadis. Identification and nomenclature of plant species were according to Täckholm (1974) and Boulos (1995; 1999; 2000; 2002; 2005).

Forty eight surface soil samples, 0-30 cm depth and 2kg each were collected from W. Feiran, W. Zaghra and W. Me'ar near the main trunk of trees at different sites (*Table 2*) to characterize the soil condition that support *M. peregrina* populations. Three soil samples were collected from each population for physical and chemical analysis. Physical parameters included particle size distribution and soil texture whereas chemical analyses included soil pH and electric conductivity (EC).

By using nets and carefully shaking the *M. peregrina* tree branches, seeds from *M. peregrina* trees growing at W. Feiran, W. Me'ar, and W. Zaghra were collected for germination and propagation experiments at the Suez Canal University (SCU, Ismailia, Egypt) botanical garden.

Table 2. *List of associated species with M. peregrina populations at different sites*

Family	**Species**
Amarnthaceae	*Aerva javanica* (Burm. f.) Juss. ex Schult.
Asclepiadaceae	*Solenostemma arghel* (Delile) Hayne
Capparaceae	*Capparis sinaica* Veill.
	Capparis spinosa L.
Chenopodiaceae	*Anabasis articulata* (Forssk.) Moq.
	Haloxylon salicornicum (Moq.) Bunge ex Boiss.
Cleomaceae	*Cleome droserifolia* (Forssk.) Delile
Compositae	*Artemisia judaica* L.
	Iphiona scabra DC.
	Launaea spinosa (Forssk.) Sch. Bip. ex Kuntze
	Pulicaria arabica (L.) Cass.
Cucurbitaceae	*Citrullus colocynthis* (L.) Schrad.
	Cucumis prophetarum L.
Euphorbiaceae	*Chrozophora oblongifolia* (Delile) Spreng.
Labiatae	*Otostegia fruticosa* (Forssk.) Penz.
	Teucrium polium L.
Leguminosae	*Acacia tortilis* (Forssk.) Hayne
	Retama raetam (Forssk.) Webb & Berthel.
	Senna italica Mill.
	Zilla spinosa (L.) Prantl
Resedaceae	*Ochradenus baccatus* Delile
	Reseda sp.
Solanaceae	*Hyocyamus muticus* L.
Zygophyllaceae	*Fagonia mollis* Delile
	Peganum harmala L.

Variation in phenotypic traits, germination and early-life growth

Vegetative parameters including crown cover, diameter, height, and average seed number/fruit and weight for each population was also measured (*Table 1*) to asses the variation in phenotypic traits between studied populations. Seeds of *M. peregrina* germinate well without any pre-treatment. So, a total of 2788 seeds from forty-seven trees representing different sampled populations were sown directly on Fafard mix no.3B (Canadian Sphagnum Peat, Vermiculite, Perlite, and Processed Pine Bark.) and placed in a greenhouse. Germination percentage and variation between sampled populations were assessed.

A total of 1302 *M. peregrina* seeds representing thirty-two tree grown at Wadi Fieran (165 seeds from five trees) and Wadi Zaghra (1137 seeds from twenty-seven trees) were planted in sand-Peat Moss 1:1 mixture and placed under open-air conditions in SCU botanical garden during the growing season (March and April). Germination percentage was assessed for every tree and the mean for every population and overall mean were calculated. Early-life growth parameters including height, number of branches, and vitality of 10 seedlings were measured every two weeks for six months. Variation in germination percentages and growth parameters between trees and populations were assessed.

Using MINITAB release 14 computer software, One Way ANOVA was used to test the variation between groups. As the ANOVA procedure depends on data being at least a reasonably close approximation to a normal distribution, the Anderson-Darling test was used to allow the possible conclusion that the departures from normality are detectable or not. Bartlett's or Levene's test was used to test for variance homogeneity. Bartlett's test was used when the data come from a normal distribution and Levene's test was used when the data come from continuous, but not necessarily normal, distributions. Kruskal-Wallis was used as an alternative to ANOVA with data sets that failed that test for normality and/or homogeneity of variance.

Variation in early life-history fitness

Survivorship of any plant in such harsh desert environment depends on its fitness to this environment. Since fitness is the probability of a plant to survive until the reproductive age and gives offspring, it is a net function of the total number of seeds that the mother plant produces, the viability of the produced seeds, the probability of germination and producing seedlings, and the probability of these seedlings to grow ahead of seedling (juvenile) stage and get established (adult). Abd El-Wahab (1995) revealed that the seeds of *M. peregrina* is almost hundred percent viable. As a result, the viability does not contribute to differentiate between populations in their fitness. The net number of seeds been produced by a tree could be, logically, calculated through estimating the average number of seeds each fruit has, multiplied by the total number of that fruits a tree produce. Unless been in fenced enclosures, practically in highly palatable seeds, the total number of fruits been produced by a tree could not be estimated. Meanwhile, the average number of seeds per fruit is still a good indicator for the overall seed production and that is what we adopted here.

Growth parameters like number of branches, height, and vitality reflects the healthiness of the tree and hence refers directly to establishment probability. As *M. peregrina* seeds are highly palatable, its weight (as it reflects its size and storage material content) represents a major factor in determining the risk of been eaten. The higher weight of seeds the higher probability of been eaten, i.e. low establishment probability. Therefore, six measured life-history parameters (average number of seeds/fruit, seed weight, germination percentage, height, number of branches, and vitality) were subjected to principal component analysis to develop a smaller number of artificial variables (principal components) that will account for most of the variance in the estimated fitness in case there is some redundancy in those variables. As long as *Moringa peregrina* seeds have no innate dormancy, the germination rate may reflect the total fitness including female (production of ovules) and male fitness (fertilization of produced ovules).

The transformed data (divided by the maximum measured value) of average number of seeds/fruit and seed weight, germination percentage (in greenhouse and open-air), and growth parameters (height, number of branches, vitality) were used to construct a fitness index for the Early life-history as follows:

- Fitness = average number of seeds/fruit X seed weight X germination (greenhouse & open air) X height X number of branches X vitality
- Statistical analyses of fitness traits and fitness index were performed using MINITAB release 14 computer program. One-way ANOVA or Kruskal-Wallis was used to test for significant differences between populations. Kruskal-Wallis test was used when ANOVA could not be used due to a violation of its assumptions, normality and/or variance homogeneity.

Results

Ecological status assessment

Three Wadis in South Sinai known to support *M. peregrine* populations were surveyed. Sixteen sites containing a total of 197 *M. peregrina* trees were recorded (*Table 1*). The largest population (N = 40) was recorded in W. Fieran (site 3). Two isolated trees were recorded, one in site 10 in W. Zaghra and another in site 11 in W. Fieran. The most noticeable field observation was that almost all reproductive trees grow on south facing slopes and crevices of metamorphic rocks. At the same Wadi, trees growing on north facing slopes do not flower. This observation may indicate the importance of the light and temperature as reflected by slope exposure as a limiting factor for growth, flowering and fruiting by *M. peregrina*. Most *M. peregrina* populations grow on cliffs and at the base of hills with very rugged topography. The elevation of these hills ranges between 300 to 800 m above sea level. The narrow distribution of *M. peregrina* is due to over-grazing (the seeds are highly grazed by goats and sheep), soil moisture content and over-cutting.

Twenty five species were recorded as commonly associating species to *M. peregrina* in the surveyed sites. Associating species (*Table 2*) include *Acacia tortilis, Aerva javonica, Capparis spinosa, Ephedra alata, Hyoscyamus muticus, Peganum harmala,* and *Pergularia tomentosa*.

Soil physical analysis showed that surface soils of the different sites supporting *M. peregrina* populations are characterized by very narrow (non-significant, *Table 3*) variation in soil texture with more or less neutral to slightly alkaline sandy soil (pH = 6.5 – 7.9 and sand fraction = 62.7 – 96.8 %) and with low salinity level (EC = 0.4 – 15.4 mS/cm). Pearson linear correlation test showed highly significant positive correlation between EC and Clay content (CC = 0.582, P = 0.001) and negative one between silt and sand fractions (CC = -0.989, P = 0.000).

Table 3. *Summary of soil analysis results at different sites*

Wadi		**pH^{NS}**	**EC^{NS} (mS/cm)**	**Clay^{NS} (%)**	**Silt^{NS} (%)**	**Sand^{NS} (%)**	**Soil Texture**
Feiran	**Min**	6.87	1.51	2.72	2.60	89.22	Sandy
	Max	7.72	3.71	3.86	7.68	93.54	
	Mean	7.22	2.43	3.27	5.07	91.66	
	SD	0.35	0.96	0.44	1.80	1.60	
Zaghra	**Min**	6.47	2.02	1.46	1.97	62.70	Sandy loamy to Sandy
	Max	7.83	15.35	5.58	33.94	95.36	
	Mean	7.17	6.00	3.76	6.66	89.59	
	SD	0.37	4.07	1.15	8.78	8.66	
Me'ar	**Min**	6.94	0.42	2.62	0.61	90.36	Sandy
	Max	7.93	11.63	5.00	6.87	96.78	
	Mean	7.33	3.56	3.41	2.93	93.67	
	SD	0.30	3.48	0.73	1.92	2.19	
Total	**Min**	6.47	0.42	1.46	0.61	62.70	Sandy loamy to Sandy
	Max	7.93	15.35	5.58	33.94	96.78	
	Mean	7.24	4.37	3.53	4.84	91.63	
	SD	0.33	3.67	0.89	5.93	5.94	

Note: * = $P \leq 0.05$, ** = $P \leq 0.02$, NS = non significant variation

Human activities that threaten *M. peregrina* populations in the studied three Wadis are the over-grazing, over-cutting, and over-collection. Over-cutting and breaking down *M. peregrina* fragile branches are one of the best preferred sources for cooking and heating for most of Bedouins living near *M. peregrina* populations and especially when there are no other substitutes (e.g *Acacia tortilis*, *Anabasis articulata*, *Artemisia judaica*, and *Haloxylon salicornicum*) in very dry seasons and years like the recent ones.

Although *M. peregrina* is one of the most suffering medicinal plants from over-collection of ripened seeds for feeding livestock animals, local trade, and research, this collection has more profound effect on *M. peregrina* populations than over-grazing and over-cutting where most of the produced seeds are eaten by grazing shoats *in situ*. Off course the magnitude of the anthropogenic effect differs from population to another and from Wadi to another depending on the closeness and size of the human population size nearby the targeted *M. peregrina* population. It is higher on populations grow at W. Fieran (more than one thousand families) and W. Me'ar (two Bedouin settlements, seven-one families, Abd El-Wahab *et al.*, 2004) than that at W. Zaghra where only one Bedouin settlement (five families, Abd El-Wahab *et al.*, 2004) occurs. These human un-managed activities add on the regional natural hazards including the unpreceded prevailing aridity conditions in recent years that made most of the medicinal plants in South Sinai are considered as either endangered or vulnerable species. Although some traders import *Moringa* seeds for abroad sources, the shortage supply of local *M. peregrina* seeds at different herb shops is a clear evidence of being threatened and a reason for over-collection for trade. The seeds have been sold by herbalists under the Arabic name of "Habba Ghalia" for 30-40 LE per Kg (MPCP, 2006). Its vernacular name may reflect it scarcity and/or importance. In herbal traditional medication, being edible, its seeds are mixed with other herbs and used mainly as anti-malnutrition food (MPCP, 2006).

Phenotypic variation

Seed characters (weight and number)

Although there was variation detected between populations growing at different Wadis in the average seed weight, it was not significant. Generally, seeds collected from populations at W. Feiran (mean = 0.74g, SD = 0.11) have higher weight than that collected from populations at W. Zaghra (mean = 0.58g, SD = 012) and W. Me'ar (mean = 0.58g, SD = 058), respectively (*Table 4*). It varied from 0.61 to 0.89g for the populations growing at W. Fieran, from 031 to 089g at W. Zaghra, and from 0.33 to 0.97g at W. Me'ar. The overall mean is 0.60g (SD = 0.14). Unlike the average seed weight, number of seeds per pod showed a highly significant variation (P = 0.000) between populations and between individual. It ranged from two to eighteen in trees at W. Feiran and to twenty-three in trees at W. Zaghra.

Vegetative parameters

Tree crown cover and height showed a highly significant variation (P = 0.000) between populations and Wadis (*Table 1*). Trees at Wadi Zaghra showed the highest cover (mean = 20.81, SD = 8.01) and height values (mean = 10.30, SD = 4.93) followed by trees at Wadi Fieran (mean cover = 8.11, SD = 5.61, mean height = 3.83, SD = 2.56) and trees at Wadi Me'ar (mean cover = 6.28, SD = 3.33, mean height = 3.70, SD = 2.00). Pearson correlation coefficient showed a highly significant positive correlation between the trees crown cover and their height which is very logical, but did not show any significant correlation with the measured soil characters.

Variation in growth and fitness traits

Germination

Germination percentage of *M. peregrina* seeds planted in greenhouse was significantly higher than 55.56 %. It had highly significant difference among trees (P = 0.000) and ranged highly from 0% to 100% with an overall mean percentage of 89.7 % (SD = 17.42). Although this highly significant variation between trees together with the apparent variation in mean germination percentage in different Wadis (97.38%, 88.87%, and 80.00 % for seeds from W. Fieran, W. Zaghra, and W. Me'ar, respectively), this apparent variation between populations was not significant (P = 0.12). Pearson correlation coefficient showed a positive highly significant correlation between *M. peregrina* seed weight and germination percentage in greenhouse (P = 0.001).

Germination under the open-air condition in botanical garden at Ismailia did not show a significant variation between populations in Wadi Fieran and W. Zaghra. However, the mean germination percentage differed between the two wadis. While it was significantly higher than 70.7 % for populations at W. Zaghra, it was only higher than 42.2 % for populations at W. Fieran (*Table 4*). This open-air germination did not show significant correlation with germination in greenhouse condition (*Table 5*).

Table 4. *Growth and fitness traits*

Wadi (Location)	Pop	Avg. seeds/pod**				Avg. seed weight ^{NS}				Germination in Greenhouse ^{NS}				Avg. Germ. % in botanical garden ^{NS}				Avg. height (cm) **				Avg. # branches ^{NS}			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
W. Fieran	2	3	13	7.11	3.98	0.63	0.63	0.63	-	80.00	100.0	95.00	8.08	98.00	98.00	98.00	-	6.8	13.0	9.11	1.78	4	9	6.4	1.26
	5	2	18	5.17	4.30	0.78	0.89	0.83	0.06	85.71	100.0	98.26	4.29	54.00	100.0	79.00	23.30	5.0	12.5	6.71	1.86	4	9	6.0	1.23
	6	Bulked				0.71	0.71	0.71	-	96.00	96.00	96.00	-	-	-	-	-								
	11	Bulked				0.61	0.61	0.61	-	100.0	100.0	100.0	-	-	-	-	-								
Subtotal		***2***	***18***	***5.72***	***4.24***	***0.61***	***0.89***	***0.74***	***0.11***	***80.00***	***100.0***	***97.38***	***5.52***	***54.40***	***100.0***	***83.80***	***21.20***	***5.0***	***13.0***	***7.39***	***2.12***	***4***	***9***	***6.11***	***1.23***
W. Zaghra	7	3	23	11.12	4.78	0.44	0.76	0.56	0.10	40.00	100.0	91.47	12.90	70.00	100.0	88.80	12.21	5.0	12.0	7.59	1.16	4	9	5.76	0.92
	8	2	19	9.92	3.90	0.31	0.89	0.58	0.14	0.00	100.0	87.59	20.26	86.00	100.0	95.85	4.45	4.5	9.5	7.14	1.1	3	8	5.94	1.03
	9	2	20	9.32	4.09	0.46	0.77	0.60	0.10	0.00	100.0	88.63	16.68	50.00	100.0	88.22	12.21	5.0	9.0	7.10	0.87	4	8	5.67	0.87
Subtotal		***2***	***23***	***9.99***	***4.22***	***0.31***	***0.89***	***0.58***	***0.12***	***0.00***	***100.0***	***88.87***	***17.47***	***50.00***	***100.0***	***92.00***	***10.85***	***4.5***	***12.0***	***7.21***	***1.06***	***3***	***9***	***5.77***	***0.96***
Me'ar	12	Bulked				0.43	0.68	0.56	0.18	96.00	100.0	98.00	2.83	-	-	-	-								
	13	Bulked				0.33	0.97	0.67	0.32	0.00	100.0	66.7	57.7	-	-	-	-								
	14	Bulked				0.43	0.49	0.46	0.04	70.6	95.8	83.2	17.8	-	-	-	-								
Subtotal						***0.33***	***0.97***	***0.58***	***0.22***	***0.00***	***100.0***	***80.00***	***37.00***	-	-	-	-								
Total		**2**	**23**	**9.48**	**4.44**	**0.31**	**0.97**	**0.60**	**0.14**	**0.00**	**100.0**	**89.70**	**17.42**	**50.00**	**100.0**	**90.94**	**12.45**	**4.5**	**13.0**	**7.23**	**1.22**	**3**	**9**	**5.81**	**1.00**

Note: * = $P \leq 0.05$, ** = $P \leq 0.02$, NS = non significant variation.

Height, branching, and vitality

The height of *M. peregrina* juveniles grown in botanical garden of SCU at Ismailia under uncontrolled condition (open-air) during growing season (March – October) showed a highly significant variation between trees (*P* = 0.000) and between populations (*P* = 0.000). Although height was positively correlated with number of branches (C.C. = 0.967, *P* = 0.000), the latter had variation between trees but not populations (*P* = 0.000 and 0.201, respectively). Meanwhile, Pearson test did not detect significant correlation between the height and either germination in greenhouse or in botanical garden (C.C. = 0.063, 0.086, respectively).

Although Kruskal-Wallis test revealed a highly significant variation in measured vitality between trees and populations (*P* = 0.000 for both), Pearson coefficient did not recognize any significant correlation between vitality and other measured parameters (*Table 5*).

Table 5. *Pearson's product-moment correlation coefficients among the measured life-history characters*

Variable	Germination % in greenhouse	Germination % (open air)	Height	Branch no.	Seed number	Seed weight
Germination % (open air)	-0.062 0.741					
Height	0.063 0.737	0.086 0.645				
Branch no.	0.080 0.667	0.034 0.857	**0.967** **0.000**			
Seed number	0.212 0.252	0.107 0.566	-0.065 0.727	-0.117 0.532		
Seed weight	**0.572** **0.001**	-0.075 0.687	-0.021 0.909	0.011 0.953	-0.331 0.069	
Vitality	0.173 0.353	-0.094 0.615	-0.119 0.523	-0.189 0.308	0.327 0.073	-0.070 0.710

Early life-history fitness

Correlation coefficients among the measured life-history characters are presented in *Table 5*. Except for two sets of characters, one is related to seeds (germination in greenhouse and weight) and the other is related to seedlings (height and number of branches), all other intercorrelations were not significant (*Table 5*). PCA reduced the total set of partly intercorrelated variables to four uncorrelated principal components. The component loadings per original variable and the proportion of total variance explained by the four components are given in *Table 6*. Together, the four principal components explain 87.1 % of the total variation. The first main fitness component appears to be correlated with a combination of seedlings height and number of branches. Germination rate in greenhouse and seed weight have the highest loadings for the second fitness component while number of seeds per pod and produced seedling vitality have the highest loadings for the third fitness component. The last component is correlated with germination rate under open-air conditions.

Generally, the early life fitness index for *Moringa peregrina* populations was very low (mean = 0.06, SD = 0.03). Kruskal-Wallis test showed that the variation between populations was not significant (H = 5.94, P (adjusted for ties) = 0.203). While both are significantly higher than zero, populations at W. Zaghra showed very little higher mean fitness value (0.06, SD = 0.03) than those at W. Fieran (0.04, SD = 0.02).

Table 6. *Principal component loadings of the measured life-history characters (fitness characters). Loadings given in boldface show the highest correlation between original values and principal component scores.*

Variable	PC1	PC2	PC3	PC4
Germination %	-0.056	**-0.661**	0.323	-0.153
Germination % (open air)	-0.066	0.205	0.111	**-0.913**
Height	**-0.654**	0.078	0.235	0.089
Branch no.	**-0.668**	0.051	0.178	0.115
Seed number	0.205	0.106	**0.686**	-0.114
Seed weight	-0.1	**-0.698**	-0.216	-0.17
Vitality	0.258	-0.114	**0.529**	0.284
Eigen value	2.067	0.605	1.421	1.006
Proportion	0.295	0.229	0.203	0.144
Cumulative	0.295	0.524	0.727	**0.871**

Discussion

Climate change and its consequences present one of the most important threats to biodiversity and the functions of ecosystems. The stress on biodiversity is far beyond the levels imposed by the natural global climatic changes occurring in the recent evolutionary past. It includes temperature increases, shifts of climate zones, melting of snow and ice, sea level rise, droughts, floods, and other extreme weather events (Omann *et al.*, 2009). During the past century, annual mean temperature has increased by 0.75°C and precipitation has shown marked variation throughout the Mediterranean basin (Osborne *et al.*, 2000). This change affected the wild vegetation of South Sinai in general and resulted in rarity of trees and change in vegetation composition (Moustafa *et al.*, 2001). Unmanaged anthropogenic practices and the natural sparseness of vegetation in desert ecosystems magnify the effect of climate change and results in more fragile and sensitive ecosystem to human impacts (Batanouny, 1983).

Habitat fragmentation is considered to be one of the major threats to biological diversity (Eriksson and Ehrlén, 2001; Oostermeijer, 2003). Human activities have had a strong impact on landscape structures, often associated with a decline in habitat size, an increase in habitat isolation, altered habitat conditions and loss of plant-animal interactions (e.g. Fischer and Matthies, 1998; Kearns *et al.*, 1998). Various studied have shown that plants in fragmented populations may be smaller, have lower reproductive output and reduced seed germinability compared to non-fragmented populations.

As a multi-use economical important medicinal species, *Moringa peregrina* has experienced unmanaged anthropogenic practices (especially the over-collection of ripened seeds) beside the harsh habitat conditions resulting in a sharp decline in number and size of its populations in Sinai (Abd El-Wahab, 1995; abd El-Wahab, 2004). This reduction in the number of populations and individuals has had a negative impact on the wildlife of the region since *M. peregrina* together with *Acacia* trees and various shrub species provide an essential food source for desert wildlife. Furthermore, the decline of

M. peregrina populations has resulted in the loss of a primary natural resource for the local Bedouin communities and their domestic animals.

The data presented in this paper clearly show that remnant populations of the rare medicinal species *Moringa peregrina* have very low early stage fitness estimated as an index of maternally affected life-history characters, represented by seed number and weight, germination rate under greenhouse and open-air conditions, and seedlings characters including height, number of branches, and vitality. All of the studied populations could be considered small ones, meanwhile Zaghloul *et al.* (in preparation) revealed that these populations have reduced genetic diversity. This may suggest that the deteriorated environmental circumstances, which the maternal plants in small populations encounter, affect the performance of their offspring negatively. Several previous studies (e.g. Schaal, 1984; Roach and Wulff, 1987) showed that environmental stress on maternal plants is carried over to the progeny, especially in the first stages of their development. Also, several previous works (e.g. Ouborg *et al.*, 1991; Oostermeijer *et al.*, 1994) demonstrated a positive relationship between population size and variation in fitness components for rare perennial plant species. Similar results were obtained in *Acacia tortilis* subspecies *raddiana* populations growing in the same habitats (Zaghloul, 2008).

Zaghloul *et al.* (in preparation) demonstrated that *Moringa peregrina* populations experience real selfing (16%) and open-pollinated seeds are a product of both selfing and outcrossing events. The potential for maternal plants in small populations to invest in offspring may also be reduced by inbreeding depression, or because they are, just by chance, a poorly performing sample of survivors from the previous larger populations, a phenomenon known as demographic stochasticity (Menges, 1991). However, it is not likely for the long-lived *Moringa peregrina* (unpublished data) that there has been a history of inbreeding. So, it is most likely that the very low maternally affected fitness is due to environmental stress (including threats imposed by contemporary prevailing drought and anthropogenic practices) on the remaining populations.

The results of current study showed a highly significant variation ($P = 0.000$) between populations in number of seeds per pod, crown cover, and height of trees (*Table 1*). This variation could be attributed to very low observed heterozygosity ($H_o = 0.04$, Zaghloul *et al.*, in preparation) that is recorded in these populations that the reduced heterozygosity does not only lower individual fitness, but may also increase the amount of variation among progeny since 'developmental homeostasis' is disturbed in homozygotes (Mitton and Grant, 1984; Mitton, 1989).

In conclusion, current threats and the prevailing harsh habitat conditions are the main causes for the need of the species conservation and development of a plan for its sustainable management in the Sinai. Direct protection is urgently needed to stop further deterioration of *M. peregrina* populations and to improve their ability to maintain or improve population numbers.

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COMPARATIVE ANALYSIS OF THE RELATIONSHIP BETWEEN PHENOLOGICAL PHENOMENA AND METEOROLOGICAL INDICATORS BASED ON INSECT AND PLANT MONITORING

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Abstract. Climate change is one of the biggest environmental problems of the 21st century. The most sensitive indicators of the effects of the climatic changes are phenological processes of the biota. The effects of climate change which were observed the earliest are the remarkable changes in the phenology (i.e. the timing of the phenophases) of the plants and animals, which have been systematically monitored later. In our research we searched for the answer: which meteorological factors show the strongest statistical relationships with phenological phenomena based on some chosen plant and insect species (in case of which large phenological databases are available). Our study was based on two large databases: one of them is the Lepidoptera database of the Hungarian Plant Protection and Forestry Light Trap Network, the other one is the Geophytes Phenology Database of the Botanical Garden of Eötvös Loránd University. In the case of butterflies, statistically defined phenological dates were determined based on the daily collection data, while in the case of plants, observation data on blooming were available. The same meteorological indicators were applied for both groups in our study. On the basis of the data series, analyses of correlation were carried out and a new indicator, the so-called G index was introduced, summing up the number of correlations which were found to be significant on the different levels of significance. In our present study we compare the significant meteorological factors and analyse the differences based on the correlation data on plants and butterflies. Data on butterflies are much more varied regarding the effectiveness of the meteorological factors.

Keywords: *climate change, phenophases, meteorology, correlation, Lepidoptera*

Introduction

Range and spatial and temporal pattern of the species making up the biosphere has been continuously changing since life began on Earth. The most important pattern generating factor of this global change is weather and its seasonal changes. Earlier, the change of the biota in geological scale could be considered to be a natural process, however, in the ecological and climatological changes of the last some thousand years the effects related to human activity are more and more significant. Direct and indirect effects of the climate change on terrestrial and marine ecosystems could already be observed in the last decades – on the level of individuals, populations, species, biomes and the global biosphere as well. Climate change is one of the most important ecological problems of this century (IPCC, 2007). It is of great significance because it affects the living conditions of the whole global society (Harnos et al., 2008) so it determines its sustainability as well. The changeability of the climate, that is the lack of climatic stability (and its degree) in longer periods, is determinant for the state and change of state of all earthly ecosystems (Hufnagel and Gaál, 2005; Őszi et al., 2006; Ladányi and Hufnagel, 2006; Szenteleki et al., 2007; Erdélyi, 2008; Gaál, 2008; Ladányi, 2008). The different natural and human-influenced ecosystems show heterogeneity regarding their sensitivity to the effect (Sipkay et al., 2007, 2008; Hufnagel et al., 2008). Ecosystems as systems capable of regulation do not only endure effects passively but they react to those with adaptation of different degree and type, feedback and regulation (Drégelyi-Kiss, 2008).

The effect of climate change which was observed the earliest and has been systematically monitored later as well is the remarkable changes in the phenology (i.e. the timing of the phenophases) of the plants and animals. Despite the fact that not enough is known about which environmental factors induce the beginning of the phenophases and affect the reproduction cycles in case of individual species, it is known, that not only the changing weather (due to the higher CO_2 concentration) but the CO_2 level itself can significantly affect e.g. the blooming of some plants (Houghton, 1995). Several biologists have already studied the phenological changes all over Europe, and it was observed that certain species started budding 5-6 days earlier than usual, while e.g. autumn colouring started 4-8 days later (Menzel, 1999). Abu-Asab and his colleagues (2001) studied the changes in the beginning of blooming over 21 years (between 1970 and 1990) and their results show that the majority of the trees (regarding 100 species from 44 angiosperm families) started blooming 3-5 days earlier at the end of the period than at its beginning. Earlier beginning of blooming is also proven by the research results of Primack and his colleagues (2007), according to which rise in the mean temperature by 1 °C between February and May results in blooming approximately 4 days earlier in Massachusetts (USA), so plants bloom nowadays 11 days earlier on average than a century before. However, researches by Sherry et al. (2007) show that only those species start blooming earlier which bloom before the summer heat, the blooming of the other ones is definitely delayed. In the American tall grass prairies early species bloom 7.6 days earlier on average and late species 4.7 days later, so in the middle there is a gap in the seasonality of the community's blooming dynamics. It can be seen from the above that out of the basic ecological phenomena, climate change modifies the seasonal community dynamics and one of its important factors: the phenology of species, the most significantly (Schwartz, 2003; Vadadi et al., 2008).

In our present study, which is based on the development of our researches last year (Eppich et al., 2009), we wanted to examine the connection between weather conditions and phenology and compare our studies on geophytes with data on insect phenology.

In our research we set three main goals again:

1. To explore based on some suitably chosen years which role frequency distributions of the meteorological parameters of the given period play regarding the phenological patterns.
2. To survey with the help of correlation analyses, which meteorological parameters have what kind of influence on the phenological behaviour of the individual indicator plants and insect species.
3. To compare the effects observed in the case of plants and insects and find out whether they show similar phenomena (i.e. whether synchronicity can be assumed in their changes) or there are differences, which can show the breaking up and reorganisation of the ecological relationships on community level.

Materials and methods

For our case study we used historical weather data series from the Hungarian Meteorological Service, Szaniszló Priszter's observations on the phenology of geophytes as well as the data on Lepidoptera from the Hungarian Plant Protection and Forestry Light Trap Network.

Meteorological data

The Hungarian Meteorological Service has published the daily data of Budapest of a 100-year period, among which you can find the daily mean temperature, the daily maximum and minimum temperature, the amount and type of daily precipitation and the daily sunshine duration. In order to complete these, radiation values were also calculated (according to Gábor Szász, 1968).

Phenological database on geophytes

Szaniszló Priszter, the former director of the Botanical Garden of the Eötvös Loránd University had been observing and recording the beginning of three characteristical phenophases of about 200 plant species, mainly geophytes for approximately 40 years during the last decades of the twentieth century (Priszter, 1960 – 2000; Priszter, 1974; Isépy and Priszter 1972; Priszter and Isépy, 1974). Priszter's data were substituted for day serial numbers in the individual years. These data describe on which days the observed geophytes budded, bloomed and withered. Our database was constructed from these data and it contains Latin names of the species and day serial numbers of the three phenological events for each examined year.

Hungarian Plant Protection and Forestry Light Trap Network

On the initiative of Tibor Jermy, member of the Hungarian Academy of Sciences, the organizing of the Hungarian light trap network began in 1952, on plant protection stations, in agricultural institutions and forestries. The Hungarian light trap network is equipped uniformly with Jermy-type light traps. Several light traps are operating covering the whole country, from which samples are collected by daily emptying. This way we possess daily flight data of several decades on hundreds of insect species. However, the data series of the light trap network are burdened by numerous errors, missing data, and the filtering, correction and homogenization of these is a serious biomathematical problem. All things considered, the database of the light trap network provides a unique opportunity for the phenological researches on Lepidoptera (Nowinszky, 2003). For the present study we prepared homogenized, interpolated daily values after error filtering, adjusted by a space-time window, from the united data series of suitably selected light traps. As phenological event we selected the day of the data series obtained this way on which the cumulated sum of the frequency data first exceeds 50% of the annual sum.

Used meteorological indicators and their evaluation

The meteorological indicators have been calculated from the end of August in the previous year (from the 240th Julianus day of the year) till the beginning of the phenological event.

The following derived meteorological parameters have been calculated:

1. average of daily global radiation (J/m^2),
2. average of daily mean temperature,
3. average of daily maximum temperature,
4. average of daily minimum temperature,
5. precipitation amount,
6. sunshine duration,
7. daily average of sunshine duration,
8. number of days with precipitation (including trace of precipitation),
9. number of days with real precipitation (excluding trace of precipitation),
10. sum of mean temperature > 10 °C,
11. sum of mean temperature > 9 °C,
12. sum of mean temperature > 8 °C,
13. sum of mean temperature > 7 °C,
14. sum of mean temperature > 6 °C,
15. sum of mean temperature > 5 °C,
16. sum of mean temperature > 4 °C,
17. sum of mean temperature > 3 °C,
18. sum of mean temperature > 2 °C,
19. sum of mean temperature > 1 °C,
20. sum of mean temperature > 0 °C,
21. average of daily temperature fluctuation (maximum-minimum),
22. relative deviation of precipitation for days with precipitation,
23. number of frost days,
24. sum of nonnegative daily mean temperature after the last frost day till the day of the phenological change.

Using these meteorological indicators, analyses of correlation have been carried out on the phenophases in our geophytes' phenology database and the light trap data series on Lepidoptera for each year of examination. For our work the statistical software package PAST (Hammer et al., 2001) has also been used (Dede et al., 2009).

To describe the significance of each meteorological indicator the G index was introduced. In its numerator the number of the correlations which are significant at 5% significance level is summed up with double weight, while the number of the correlations which are significant at only 10% significance level is simply summed up, and in the denominator there is twice the total number of the studied species (maximum correlation).

From the meteorological data series the yearly frequencies of each characteristical meteorological indicator were also calculated for the periods lasting from the end of August in the previous year till the end of August in the current year, and their distributions were plotted in order to find out the degree of the variability between the years.

Results and discussion

The phenological role of the various meteorological parameters we analyzed and their importance are expressed by the G index. It was calculated for both the geophyte and the Lepidoptera databases (*Table 1*).

Based on these data it can be stated that Lepidoptera data show significantly higher correlations on average with meteorological parameters than phenological data of the geophytes do. This is not surprising because Lepidoptera, attracted by light, are much more exposed to the effects of climate and vegetation than geophytes, which possess significant nutrient reserves and accumulated water. At the same time it is an extremely interesting and consistent result that the most important factor regarding both Lepidoptera and plants proved to be No. 21: the average of daily temperature fluctuation. This is surprising because models used in phenology generally yield good results rather with parameters related to heat summation or mean temperature. Parameter No. 6, the sum of sunshine duration is also important for both groups. It can be also stated that parameters No. 5, 7 and 22, namely precipitation amount, daily average of sunshine duration and relative deviation of precipitation do not play an important role either in the case of Lepidoptera or in the case of plants. The ineffectiveness of the variables describing precipitation conditions is not surprising because it is a characteristic of bulbous plants that they are capable of tolerating the lack of precipitation due to their storage organs so they are not affected by precipitation conditions as much. However, the average of sunshine duration and the average of temperatures above 10 °C do not seem to be effective in case of our plants. This can be surprising because sunshine and heat usually prove to be important factors when examining phenological phenomena.

A striking difference can be seen concerning indicator No. 23 (number of frost days) because it is very important in the case of geophytes but in the case of Lepidoptera it seems to play almost the least significant role. It is easy to understand that frost has a significant effect on geophytes, while in the case of flying insects it is not really important in itself.

Examining 93 geophytes in our earlier studies (Eppich et al., 2009 a, b) it was recognizable that the effect of several factors differs per phenophase. Apart from the above the appearance of the first buds can also be affected by the average daily maximum temperature (met3) and the average daily mean temperature (met2), which is reasonable since this is obviously in connection with spring warming. However, the date of blooming is rather affected by the average daily minimum temperature (met4) and global radiation (met1). This can be caused by the fact that the blooming of already developed buds can be limited by low temperature at night or early in the morning. The withering date of flowers, however, is often influenced by a factor that almost never has an effect on the previous two phenophases. This is the number of days with real precipitation (met9), the importance of which seems to precede even that of the number of frost days (met23) in this case. This latter observation is also easy to understand because in case of withering, temperature conditions are less dominant than precipitation conditions.

Table 1. *G indices of meteorological indicators in the correlation analyses of Lepidoptera and geophytes. G indices above 50% are highlighted in bold, the 6 largest G indices per group are underlined*

Indicators	Lepidoptera	Geophyton			
G-index	**total**	**total**	**first bud**	**blooming**	**withering**
met01	0.884	0.287	0.280	**0.333**	0.247
met02	0.750	**0.332**	**0.462**	0.344	0.188
met03	0.755	**0.299**	**0.452**	0.306	0.140
met04	0.709	**0.358**	0.435	**0.425**	0.215
met05	0.493	0.043	0.043	0.043	0.043
met06	**0.921**	**0.296**	0.371	0.290	0.226
met07	0.595	0.045	0.059	0.027	0.048
met08	0.779	0.167	0.344	0.091	0.065
met09	0.727	0.265	0.156	0.280	**0.360**
met10	0.859	0.124	0.167	0.070	0.134
met11	0.868	0.147	0.210	0.075	0.156
met12	0.875	0.169	0.247	0.091	0.167
met13	0.889	0.185	0.263	0.102	0.188
met14	0.896	0.181	0.274	0.102	0.167
met15	0.905	0.179	0.280	0.102	0.156
met16	0.909	0.186	0.285	0.108	0.167
met17	**0.914**	0.194	0.285	0.113	0.183
met18	**0.918**	0.181	0.274	0.113	0.156
met19	**0.923**	0.176	0.274	0.108	0.145
met20	**0.925**	0.176	0.285	0.097	0.145
met21	0.948	0.835	0.930	0.796	0.780
met22	0.066	0.052	0.059	0.054	0.043
met23	0.155	**0.543**	**0.710**	0.624	0.296
met24	0.666	0.174	0.183	0.172	0.167

Thus the six most important factors in case of our plants are indicators No. 2, 3, 4, 6, 21 and 23, while in case of Lepidoptera these are indicators No. 6, 17, 18, 19, 20 and 21. Accordingly, apart from the daily temperature fluctuation and sunshine duration the average daily mean, maximum and minimum temperatures are dominant in case of plants, while in case of Lepidoptera instead of these latter ones rather the sum of mean temperatures above 1, 2 and 3 °C are significant. In case of our plants 3 °C proved to be the most adequate threshold value for heat summation, in case of Lepidoptera it was 0°C. However, it can be seen that daily temperature fluctuation is more important in all cases than the approaches to heat summation.

In case of Lepidoptera we classified the meteorological parameters according to their correlations with the species. The result can be seen on *Figure 1*.

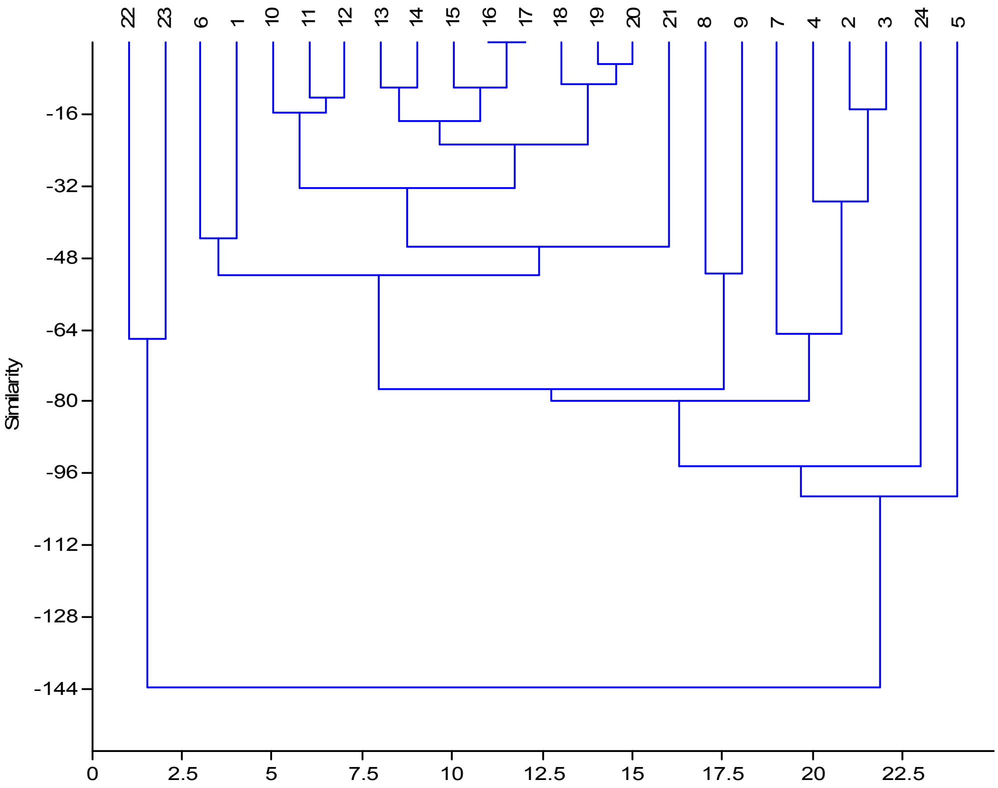

Figure 1. *The classification of meteorological parameters according to their correlations with Lepidoptera species (HC, paired group algorithm, Euclidean distance)*

It can be observed that there are numerous Lepidoptera species that correlate well with almost all parameters and a smaller group with nearly none of them. At the same time there is a well definable group of Lepidoptera for which rather parameters No. 1, 2, 3, 4, 6, 8, 9, 21, 23 are important, while for another circle instead of these parameters No. 13, 14, 15, 16, 17, 18, 19, 20, 21 are important. These correlation patterns require further analyses.

Since daily temperature fluctuations seemed to be the most important parameters, we considered it worthwhile examining their annual frequency distribution more thoroughly (*Figure 2*).

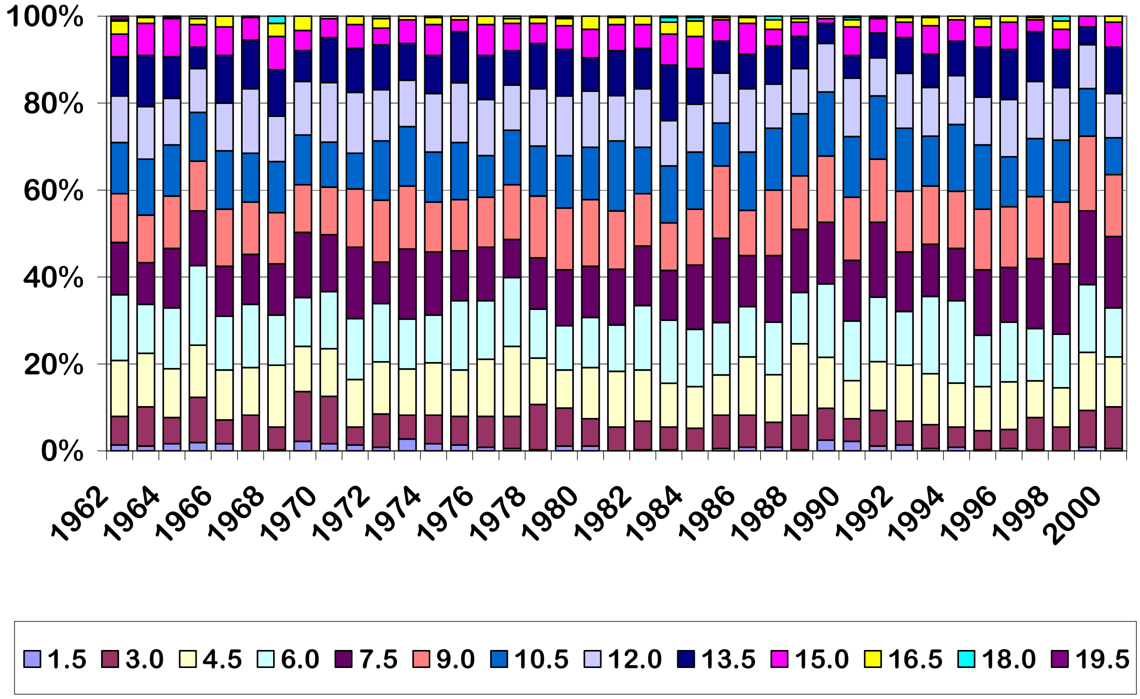

Figure 2. *Frequency distributions of values of daily temperature fluctuation in the years of examination*

Based on *Figure 2* it can be stated that no obvious tendencies can be observed regarding this factor during the examination period, however, it is noticeable that in the second half of the examination period there are several years with greater fluctuations. Examining other factors this phenomenon was not remarkable, which draws attention to the fact that greater attention should be paid to the question of daily temperature fluctuation in climate change researches.

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Electronic information source

Hungarian Meteorological Service: daily meteorological data of Budapest in the 20th century on the home page of the Hungarian Meteorological Service
http://met.hu/pages/climate/bp/Navig/Index2.htm

SEASONAL AND TEMPORAL VARIATION IN SOIL MICROBIAL BIOMASS C, N AND P IN DIFFERENT TYPES LAND USES OF DRY DECIDUOUS FOREST ECOSYSTEM OF UDAIPUR, RAJASTHAN, WESTERN INDIA

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Abstract. The soil microbial biomass of soil is being increasing recognized as sensitive indicator of soil quality. Its knowledge is fundamental for sustainable environment management. The soil microbial biomass C, N and P were studied in four different land uses of dry tropical forest of Udaipur, Rajasthan, Western India to assess the influence of abiotic, physico-chemical variables and difference in different land uses (mixed forest, butea plantation, grassland and agricultural lands) on the seasonal variation in soil microbial biomass. Microbial biomass C, N and P were highest during rainy season and lowest during winter in all the four different land use with the exception of microbial N which was lowest in summer in mixed forest and butea plantation. Microbial biomass C and N were shown to be significantly correlated to the abiotic and physico-chemical variables of the soil, such as soil temperature, relative humidity, soil moisture, organic C, total N, clay, and pH. Present study clearly shows that land use has a significant effect on microbial biomass C, N and P in soil by altering natural soil characteristics under the same ecological conditions.

Keywords: *Soil microbial biomass, soil nutrient pool, dry tropical forest, land use type, soil*

Introduction

Soil organic matter is an important components of soil quality and productivity; however, its measurement alone does not adequately reflect change in soil quality and nutrient status (Bezdicek et al., 1996). Measurement of biologically active functions of organic matter, such as microbial biomass C, N, P and potential C, N and P mineralization, could better reflect changes in soil quality and productivity. Soil microbial biomass is an important parameter linking the plants to soil. Soil microbial biomass comprises about 2–3% of total organic carbon in the soil and has been recognized as an important source of nutrients to plants because of its fast turnover. The soil microbial biomass is the labile pool of organic matter (Jenkinson and Ladd, 1981) and act as both source and sink of plant nutrients (Singh *et al.* 1989). It plays a crucial role in nutrient cycling and its importance in soil fertility and nutrient concentration is well recognized. Influence of environmental factors to microbial population and microbial biomass plays an important role in nutrients cycling in an ecosystem. The cycling of nutrients in soils of forest ecosystems is, to varying degrees, dependent on the energy supply to and through the soil biota.

Review of literature

The soil microbial biomass is an important labile pool of C, N, and P and fluctuations in its size and activity can significantly influence crop productivity (Rosswall and Paustian, 1984; McGill et al., 1986). Soil physico-chemical characteristics also has a great impact on microbial biomass and microbial activity and can be used to measure soil quality (Parr and Papendick, 1997) but it may take years for these parameters to make significant changes in soil. Changes in the microbial population in response to variation in soil conditions (Moisture, Organic C, nutrients, temperature and pH) have serious implication for nutrient cycling with microorganisms acting as a source and sink of nutrient. Soil biological and biochemical changes are very sensitive to small changes in soil conditions (degradation, erosion) and thereby give more accurate and immediate information in soil quality because soil microbial activity has a direct influence in ecosystem stability and fertility (Smith and Papendick, 1993). Insam et al. (1989) also proposed that the ratio of microbial biomass to total organic carbon in a soil might serve as a quantitative indicator of carbon dynamics in the soil. Climatic seasonality has been reported to influence microbial population and soil microbial biomass (Schimel et al., 1994) either directly by influencing microbial response to soil changes or in directly by influencing by plant metabolism.

Information on soil microbial biomass in different forest ecosystems have been reported by several workers (Srivastava and Singh, 1991; Billore et al., 1995; Joergensen et al., 1995; Arunachalam et al., 1996; Mendham et al., 2002; Lee and Jose, 2003) but information on seasonal changes in the microbial biomass in an annual cycle in forest ecosystem is limited (Diaz-Ravina et al., 1995; Arunachalam and Arunachalam, 2000). Therefore, the present study was undertaken to evaluate the seasonal fluctuation in the microbial C, N, and P and the influence of abiotic, physic-chemical variables on the microbial biomass (C, N, and P) in a dry tropical deciduous forest of Udaipur, Rajasthan, Western India.

Materials and methods

The study site is located at 23°3' – 30°12' N longitude and 69°30' – 78°17' E latitude in Aravally hills a distance of 70 km from Udaipur city, Rajasthan at an altitude ranging from 575 to 585 m above sea level. There are three distinct seasons per year: winter (November to February), summer (April to mid-June), and a rainy season (mid-June to mid- September). The months of October and March are transitional periods and are known as autumn and spring, respectively. The climate of Rajasthan is tropical with a maximum of 46.3 °C and a minimum of 28.8 °C temperature during summers. Winters are a little cold with the maximum temperature rising up to 26.8 °C and the minimum dropping to 2.5 °C. The average annual rainfall of the area is less than 400 mm.

The study was conducted year August 2008 to July 2009. Four experimental different land use types are selected for this study (mixed forest, butea plantation, grassland and agricultural land) which more or less exhibit the same ecological conditions. The elevation of forest land approximately 150m and average slope is 55% and its dominated by *Tectona grandis, Miliusa tomentosa, Lannea coromondica, Annona squamosa*, *Anogeissus latifolia*, *Wrightia tinctoria, Aegle marmelos*, *Boswellia serrata*, *Butea monosperma*, *Holoptelea integrifolia*, *Sterculia urens, Acacia Senegal.* The elevation of butea plantation is approximately 100m and slope is 45% and its dominated by *Butea monosperma, Lannea coromondica.* The elevation of Grassland and agricultural land is approximately 65m and slope is 20%. The major annual crops alternatively cultivated in the agricultural land include *Saccharum officinalae*, *Zea mays, Cicer aurentum.*

Soil is alluvial, sandy loam type of present study site. The soil is well drained with yellowish brown to deep medium black in colour. The soil samples were collected from the upper layer of 0–10 cm in depth from the four different land use types (mixed forest, butea plantation, grassland and agricultural land) for the estimation of microbial biomass. The soil samples were sieved (<2 mm) to remove stones, coarse and roots and were kept at room temperature for a day. Three replicates were collected every month from each site for the estimation of microbial biomass (C, N and P). Microbial biomass (C, N and P) were determined by fumigation extraction method (Anderson and Ingram, 1993). Microbial biomass C was determined by modified Walkley Black method and calculated by using Vance et al. (1987):

$$\text{microbial C} = \text{KEC} \times 2{:}64$$

Microbial biomass N was determined by microkjeldahl method (Bremner and Mulvaney, 1982) and calculated by Brookes et al. (1985):

$$\text{microbial N} = \text{KEN} \times 1{:}46$$

and microbial biomass P was determined by ammonium molybdate stannous chloride method (Sparling et al., 1985) and calculated by Brookes et al. (1982):

$$\text{microbial P} = \text{KEP} \times 2{:}5$$

Where, KEC, KEN and KEP are the difference between C, N and P extracted from fumigated and unfumigated soils.

The soil texture was analyzed by pipette method (Gee and Bauder, 1986). Soil moisture by gravimetric method; soil temperature is determined by a soil thermometer. Soil pH is determined (1:5 water suspension) by pH meter (Systronics). The bulk density of soil (g cm^3) was calculated using mass and volume. Pore space was calculated using the bulk and particle density. Soil organic C, total N and total P were estimated by the methods given by (Anderson and Ingram, 1993; Bremner and Mulvaney, 1982; Sparling et al., 1985), respectively.

Student's t-test and ANOVA are used to statistically analysed the data.

Results

Soil characteristics

The soil was sandy loamy with 50.02 – 56.54 % sand 12.7 – 19.4 % clay and 28.9 – 32.5 % silt in all the types of land use. Soil moisture ranged from 24.17 to 29.74 %, soil temperature ranged from 16.9 to 19.1 °C, soil pH 5.6 – 6.9, soil organic carbon 2.24 – 4.53 %, soil total N 0.32 – 0.57 %, total P 0.041 – 0.072 % and bulk density 0.86 – 1.23

g cm^3, C/N ratio varied from 6.5 to 8.5 across the four different types of land use (*Table 1*).

Table 1. *Abiotic variables and physico-chemical characteristics of soils in different land uses.*

	Mixed Forest	**Butea Plantation**	**Grassland**	**Agricultural land**
Abiotic variables				
Soil temperature (ºC)	18.7	19.1	18.4	16.9
Soil moisture (%)	25.28	24.17	25.76	29.74
Relative humidity (%)	53.49	53.49	53.49	53.49
Mean air temperature (ºC)	24.83	24.83	24.83	24.83
Rainfall (mm)	130.2	130.2	130.2	130.2
Soil physico-chemical variables				
Texture				
Sand (%)	53.8	56.54	55.71	50.02
Silt (%)	32.55	30.74	28.84	30.6
Clay (%)	13.65	12.72	15.45	19.38
Bulk density (g cm^3)	0.86±0.23	0.88±0.253	1.02±0.13	1.23±0.217
Pore Space (%)	67.67	66.84	61.51	53.63
Soil pH	5.2 - 5.7	5.5-6.1	6.0-7.3	6.9-7.8
Soil organic C (%)	2.36-4.28	2.24-3.78	1.81-3.42	1.35-2.53
Soil total N (%)	0.36-0.52	0.32-0.49	0.25-0.41	0.29-0.47
Soil available P (%)	0.047-0.064	0.041-0.059	0.035-0.042	0.031-0.052
C:N	6.5-8.2	7.0-8.5	6.6-8.6	7.0-7.9

Microbial C, N and P

In mixed forest, the microbial C, N and P ranged from 94.2 to 1507.8 µg g^{-1}, 78.2 to 128.3 µg g^{-1} and 39.7 to 84.4 µg g^{-1} respectively (*Fig 1*).

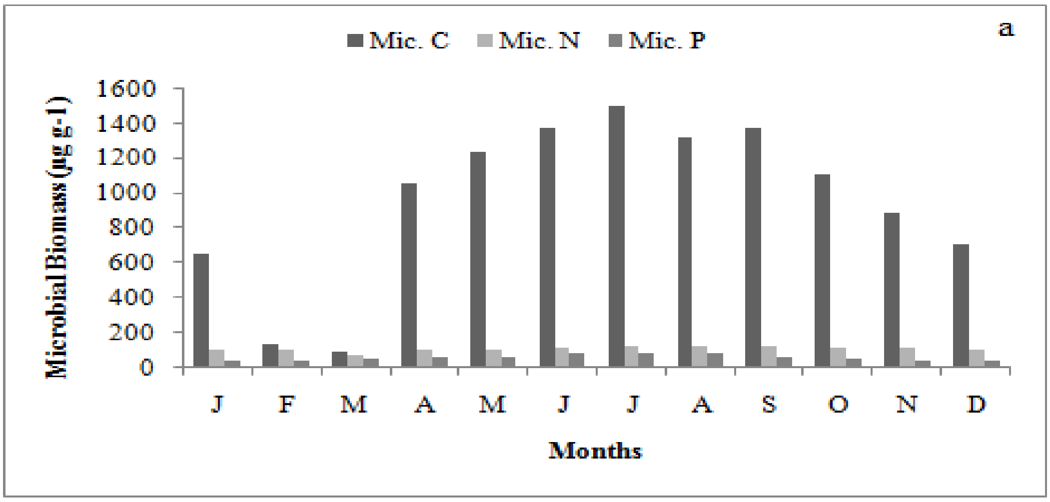

Figure 1. *Monthly variation of microbial biomass C, N and P in mixed forest*

In butea plantation, the value of microbial biomass C, N and P varied from 184.5 to 1387.7 µg g^{-1}, 69.8 to 114.2 µg g^{-1} and 32.7 to 80.1 µg g^{-1} respectively (*Fig 2*).

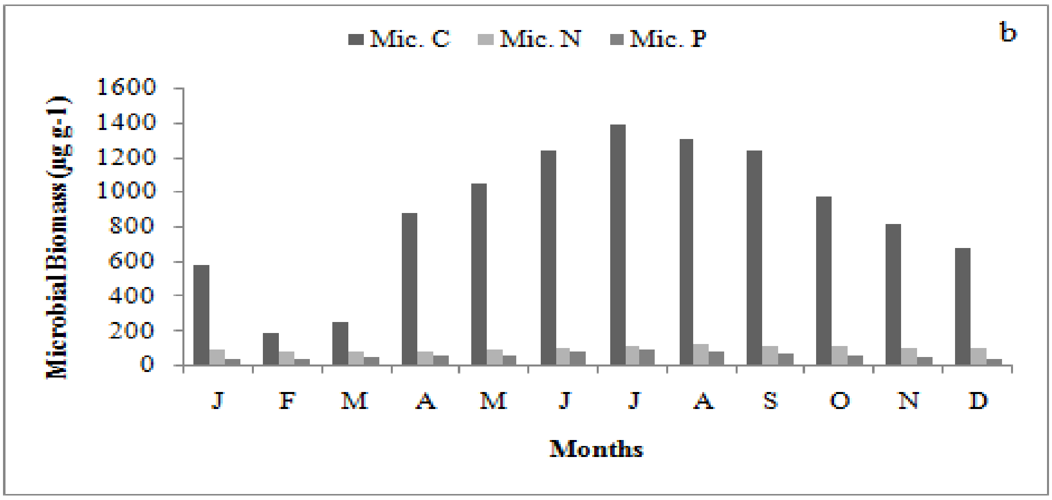

Figure 2. *Monthly variation of microbial biomass C, N and P in butea plantation*

In grassland, the val. of microbial C, N and P was ranged from 119.1 to 435.7 µg g^{-1}, 28.5 to 56.3 µg g^{-1} and 13.4 to 26.7 µg g^{-1} respectively (*Fig 3*).

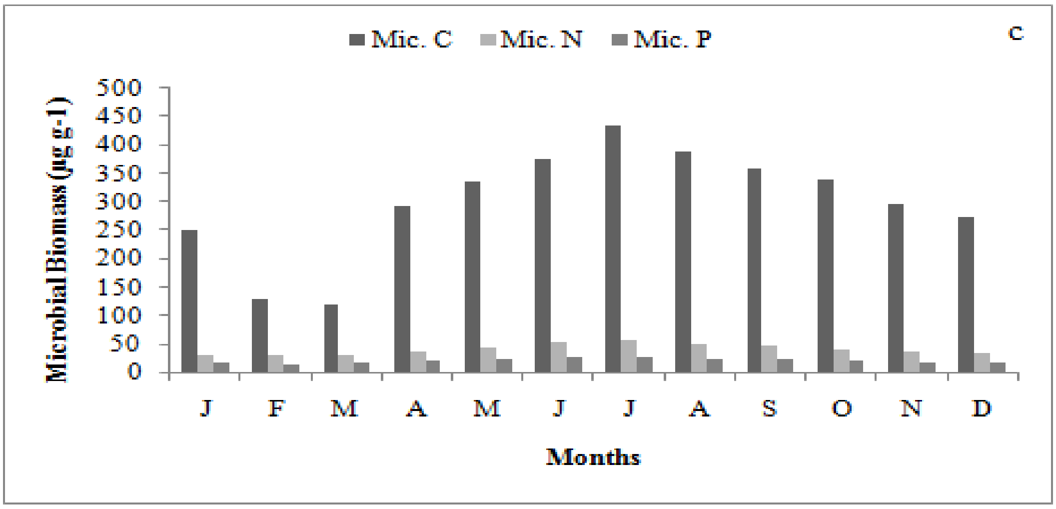

Figure 3. *Monthly variation of microbial biomass C, N and P in grassland*

In agricultural land, the microbial biomass C, N and P varied from 89.6 to 335.7 µg g^{-1}, 23.8 to 51.4 µg g^{-1} and 11.9 t0 24.9 µg g^{-1} respectively (*Fig 4*). The maximum value of microbial C, N and P was obtained in the month of July and the minimum in the month of March *(Fig. 1*). Seasonally, however, the microbial C, N and P value was recorded to be maximum during the rainy season and minimum during winter in all the types of land use with the exception of microbial N which was minimum in summer in mixed forest and butea forest. However, microbial C, N and P values are significantly higher in mixed forest than that of butea plantation, grassland, agriculture land ($P < 0.01$) (*Table 2*).

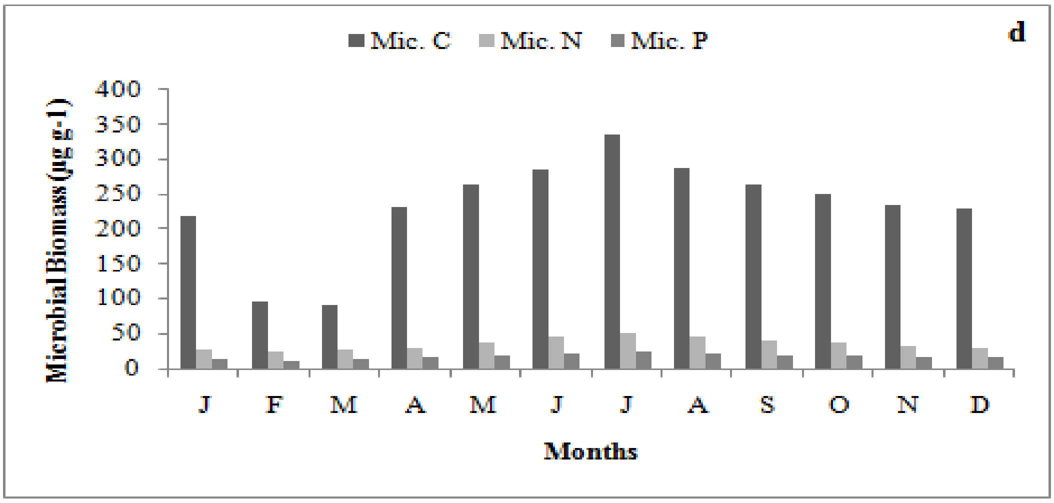

Figure 4. *Monthly variation of microbial biomass C, N and P in agricultural land*

Contribution of microbial biomass to the soil nutrient pool

In mixed forest, microbial C contributed 1.50 – 4.22 % of the total soil organic C, the maximum being contributed during rainy season and the minimum during winter season. The percentage contribution of microbial biomass N and P to total N and total P ranged from 2.06 to 3.52 % and 6.78 to 14.91 % respectively. Maximum microbial biomass N and P was contributed during rainy season and minimum during winter season. In butea plantation, the contribution of microbial biomass C to total organic C was 1.34–4.02 %, maximum value was recorded during rainy season and minimum during winter season. The percentage contribution of microbial biomass N and P to total N and total available P was 1.80–3.27 and 6.03–15.61, respectively. Maximum contribution of microbial N and P was attained during rainy and summer seasons and minimum was contributed during winter seasons, respectively.

In grassland and agriculture land, contribution of microbial biomass C to total organic C was 0.54 to 1.18 % and 0.44 to 0.86 % respectively, maximum value was reported during rainy season and minimum during winter season. The contribution microbial N and P to total N and total available P was 0.63- 1.37 and 2.46 – 5.20 % in grassland and in agriculture land 3.48 – 6.10 and 1.99 – 4.04 %. The microbial C:N and C:P ratios varied from 6.0 to 11.34 and 12.1 to 18.97 across four different types of land use.

The analysis of variance (ANOVA) indicated a significant difference in microbial biomass C between the different sampling months of summer ($P < 0.05$), rainy ($P < 0.05$), winter ($P < 0.05$) and annually ($P < 0.05$) and significant difference in microbial biomass N and P between the different sampling months of summer ($P < 0.001$), rainy ($P < 0.001$), winter ($P < 0.001$) and annually ($P < 0.001$).

Correlation coefficient between C_{mic}, N_{mic}, P_{mic}, soil temperature, soil moisture, Relative humidity, mean air temperature, rainfall, clay, Bulk density, pore space, pH, organic C, total N, available P and C:N were calculated (Table 3). The highest positive correlations were between C_{mic} and N_{mic}, C_{mic} and P_{mic}, N_{mic} and P_{mic}, relative humidity and microbial C, N and P, Soil temperature and microbial C, N and P, Soil moisture and microbial C, N and P; C_{mic} and organic C; N_{mic} and organic C; C_{mic} and total N; Organic

C and total N. On the other hand, the lowest negative correlations between organic C and pH, C_{mic} and clay, organic C and clay, bulk density and pore space (*Table 3*).

Table 2. *Microbial C, N and P (μg g^{-1}) in the soils of Mixed forest, Butea forest, Grass land and Agricultural land of Udaipur forest*

	Mixed Forest	**Butea Plantation**	**Grassland**	**Agricultural land**
Microbial C				
Summer	971.43*	851.35*	280.53*	218.15*
Rainy	1330.00*	1222.98*	380.33*	284.30*
Winter	643.91*	560.58*	236.30*	194.30*
Annual Mean	981.78±198.1	878.30±191.7	299.05±42.6	232.25±26.9
Microbial N				
Summer	106.00***	83.78***	40.78***	34.55***
Rainy	123.30***	107.85***	47.85***	42.83***
Winter	107.33***	87.98***	32.10***	27.63***
Annual Mean	112.21±5.56	93.20±7.42	40.24±4.55	35.00±4.39
Microbial P				
Summer	64.55***	58.23***	21.13***	18.00***
Rainy	70.10***	65.58***	23.40***	21.40***
Winter	43.38***	35.55***	15.28***	14.35***
Annual Mean	59.34±8.14	53.12±9.04	19.93±2.42	17.92±2.03
Microbial C:N				
Summer	9.16	10.16	6.88	6.31
Rainy	10.79	11.34	7.95	6.64
Winter	6.00	6.37	7.36	7.03
Annual Mean	8.75	9.42	7.43	6.64
Microbial C:P				
Summer	15.05	14.62	13.28	12.12
Rainy	18.97	18.65	16.25	13.29
Winter	14.85	15.77	15.47	13.54
Annual Mean	16.54	16.54	15.00	12.96
Microbial C/Organic C (%)				
Summer	2.58	2.31	0.72	0.53
Rainy	4.22	4.02	1.18	0.86
Winter	1.50	1.34	0.54	0.44
Microbial N/total N (%)				
Summer	2.26	1.90	0.89	4.72
Rainy	3.52	3.27	1.37	6.10
Winter	2.06	1.80	0.63	3.48
Microbial P/ total P (%)				
Summer	11.53	11.42	3.99	2.90
Rainy	14.91	15.61	5.20	4.04
Winter	6.78	6.03	2.46	1.99

*P < 0.05, ***P < 0.001

Table 3. *Correlation matrix (r-value) for abiotic, physical, chemical and microbiological characteristic of soil in different land uses*

Parameter	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16
C mic	1															
N mic	0.995**	1														
P mic	0.999**	0.996**	1													
Soil temperature (°C)	0.771**	0.736*	0.750**	1												
Soil moisture (%)	0.740**	0.703**	0.717*	-0.999*	1											
Relative humidity (%)	0.995**	0.999**	0.995**	0.760*	0.728*	1										
Mean air temperature (°C)	0.859**	0.813**	0.846**	-0.945**	0.934**	-0.826**	1									
Rainfall (mm)	0.189*	-0.125*	-0.203*	0.016*	-0.032*	-0.101*	0.290*	1								
Clay (%)	-0.846**	-0.815**	-0.828**	-0.992**	0.985*	-0.835*	0.966**	0.033*	1							
Bulk density (g cm^3)	-0.897**	-0.882*	-0.881**	-0.961**	0.948**	-0.899**	0.925*	-0.062	0.983**	1						
Pore Space (%)	0.899*	0.885*	0.884**	0.959**	-0.946**	0.902**	-0.924**	0.062*	-0.982*	-1.0*	1					
Soil pH	-0.889**	-0.927**	-0.891*	-0.599*	0.567*	-0.928*	0.593*	-0.212	0.676*	0.797*	-0.801*	1				
Soil organic C (%)	0.849**	0.805**	0.833**	-0.968*	0.959**	-0.821**	0.997**	0.219**	0.982**	0.944**	-0.943*	0.604*	1			
Soil total N (%)	0.565*	0.502*	0.540*	-0.934*	0.944**	-0.528*	0.899*	0.157*	0.897**	0.803*	-0.799*	0.281*	0.915*	1		
Soil available P (%)	0.027*	0.103*	0.056*	-0.571*	0.605*	0.074*	0.482*	0.105*	0.476*	0.322*	-0.315*	-0.311**	0.505*	0.808**	1	
C:N	0.251*	0.185*	0.221*	0.795**	-0.821*	0.218*	-0.689*	-0.011	-0.718*	-0.597*	0.591*	-0.001*	-0.719**	-0.937*	-0.95**	1

*P < 0.05, **P < 0.01

Discussion

The microbial C, N and P was significantly higher during the rainy season ($P < 0.05$, $P < 0.01$) and lower in winter season in both the stands with the exception of microbial N exhibiting lowest value in summer season (*Table 2*). This may be due to higher immobilization of nutrients by the microbes from the decomposing litters as decomposition rate of litters and microbial activities are at peak during this period. Further, the growth of fungi also increased during this season due to high relative humidity and thus contributing to the soil microbial biomass (Acea and Carballas, 1990).

The seasonal variation of microbial biomass C, N and P values was significant between summer–rainy ($P < 0.01$) and winter–rainy ($P < 0.01$); however, there is no significant seasonal variation between summer and winter season which may be due to unexpected rains in winter during the study period thereby increasing the microbial biomass during this season (*Fig. 3*). However, in tropical dry deciduous forest, savanna and temperate pastures peak values were recorded during early spring or summer (Saratchandra et al., 1984; Singh et al., 1989) and that of subtropical humid forest where maximum value was obtained in winter season (Arunachalam and Arunachalam, 2000) may be due to the differences in quality of litter and rainfall pattern. However, low value of microbial C, N and P in winter season may be due to low activities of microorganisms and slow rate of decomposition of litter in dry and cool period. Diaz-Ravina et al. (1995) reported that lack of water seemed to limit the microbial biomass more than temperature since lower microbial biomass contents were observed in dry period than in wet period. Several studies on soil microbial biomass reported a close relationship between soil moisture and microbial biomass (Acea and Carballas, 1990; Diaz-Ravina et al., 1995) where maximum value of microbial biomass is obtained in wet period and minimum in dry period which are in conformity with our report. Similar observations have been reported by Santruckova (1992) and Lynch and Panting (1982) in different ecosystems (forest, grassland and arable soils of Greece) and arable soil of Oxford shire, UK. The microbial C value obtained in the present study falls well within the ranges (61–2000 mg g_1) reported by Vance et al. (1987) and Henrot and Robertson (1994) for various temperate and tropical forest soils and of sub-tropical forest (978–2088 mg g_1) reported by Arunachalam and Arunachalam (2000). The microbial N value is comparable to soils of coniferous forest soils (52–125 mg g_1) reported by Martikainen and Palojarvi (1990) and evergreen forests, (42–242 mg g_1) reported by Diaz-Ravina et al. (1988), but lower than that of broad leaved deciduous forest soils (132–240 mg g_1; Diaz- Ravina et al., 1988). The microbial P value falls well within the reported range of 5.3–67.2 mg g_1 for arable land, grassland and woodland soils (Brookes et al., 1984) and 14–46 mg g_1 for sub-tropical moist forest reported by Arunachalam and Arunachalam (2000). Wardle (1998) reported in his reviewed article on soil microbial biomass dynamics that there is no consistent seasonally determined temporal patterns of microbial biomass change in tropical and warm temperate ecosystems. However, the present study in subtropical forest is contrary to his reports. The value of microbial C, N and P is comparatively higher in forest stand I situated at the foothill than in mixed forest located at higher elevation, which may be due to high soil moisture content and less exposure to sunlight and better quality of litter in former stand favouring the growth of microbes. Besides this amount of litterfall returned on the forest floor was higher than that of plantation, grassland and agriculture land. Thus, the contribution of microbial C, N and P to total soil organic C, total N and P was higher in forest mixed forest than that of plantation, grassland and agriculture land indicating microbial biomass/nutrients (C, N and P) immobilized more in mixed forest.

Both mixed forest and Butea plantation soils have significantly greater organic content and total N content in the study area when compared with grassland and agriculture land (*Table 1*). In support this finding, the highest microbial biomass C and N value were found in forest soils with highest organic C and N content. It is well known that soil organic C strongly affects the amount and activity of soil microbial biomass (Diaz-Ravina et al, 1988).

The positive and significant relation between the C_{mic} and N_{mic}, C_{mic} and P_{mic}, N_{mic} and P_{mic}, relative humidity and microbial C, N and P, Soil temperature and microbial C, N and P, Soil moisture and microbial C, N and P; C_{mic} and organic C; N_{mic} and organic C and C_{mic} and total N (*Table 3*). Our results are consistent with previously reported studies (Arunachalam and Arunachalam, 2000; Sharma etal., 2004; Wright et al., 2005). The relatively dense structure of plants and a greater accumulation of litter and fine roots in the understorey of forest and plantation may favor the growth of microbial populations and the accumulation of C in microbial biomass.

In this study, there is a significant positive relationship between soil organic C and total N, organic C and total P and total N and total P (*Table 3*). Similarly, previous studies state that if soil organic C increases, the total N increase (Manu et al., 1991; Li et al., 2007). The dynamics of N in mineral soil is closely linked to C, because most N exists in organic compounds and heterotrophic microbial biomass, which utilize organic C for energy. As a result, the microbial biomass N showed a significant positive correlation with microbial biomass C (*Table 3*). The result coincides with previous studies (Klose and Tabatabai, 1999; Arunachalam and Arunachalam, 2000; Arunachalam and Arunachalam, 2002; Sharma et al., 2004; Wright et al., 2005).

The pH of soils in, grassland and agricultural land were moderately acidic (pH 5.65) in mixed forest and butea plantation, lightly acidic (pH 6.65) in grassland and lightly alkaline (pH 7.35) in agricultural land. Relatively high values of microbial biomass C, N and P in the forest and plantation soils, compared to grassland and agricultural soil, was likely due to pH, because it showed a negative correlation with microbial biomass C and microbial biomass N (*Table 3*). The results of this study reveal that distinct plant community composition associated with 4 land use types, reflecting changes in soil pH and microbial biomass. Previous work has shown variability in microbial biomass that can be caused by alterations in soil pH (Wardle, 1992). Some authors suggest that maximum activities of soil microbial biomass occur at pH values of about 6.5 (Acosta-Martinez and Tabatabai, 2000).

The clay content of soil is known to play a role in the determining microbial biomass and activity as well as influencing the composition of microbial community (McCulley and Burke 2004). Soils with high clay content lead to more stabilization of soil organic C and higher microbial biomass (Schimel et al., 1994). In contrast, our results indicate that Cmic ($r = -0.846$, $P < 0.01$) and Nmic ($r = -0.815$, $P < 0.01$) were negatively correlated with clay content of soils. Most likely this is due to the variability in the controlling factors of microbial biomass, such as soil organic matter, management practices, and plant species composition, that may have masked the impact of clay content on soil microbial biomass.

The large variation of microbial C:N ratio (6.0 – 11.34) (*Table 2*) in the present study areas may be due to low availability of total soil N, however, the present value is close to the range reported by Martikainen and Palojarvi (1990) for various forest soils (6–9) and by Fenn et al. (1993) for chaparral soils (7–13). According to Jenkinson and Ladd (1981) C:N ratio of fungal hyphae is often 10–12 and that of bacteria usually between 3 - 5. Since C:N ratio in the present study areas are more than 5, it may be dominated by fungal community. However, several workers have reported that dominance of fungi in an acid forest soil have a significant impact on microbial C:N ratio (Swift et al., 1979; Fliessbach and Reber, 1991). The microbial C:P in the present study falls well within the reported range of 10.6–35.9 by Brookes et al. (1984), but lower than that of dry tropical deciduous forest (35.51) reported by Devi Sarjubala (2002) and sub-tropical humid forest (33.2–98.5) reported by Arunachalam and Arunachalam (2000) which may be due to high microbial biomass P in the present forest.

The contribution of microbial biomass C to soil organic C (0.44 – 4.22 %) and microbial N to total N (0.48–3.52 %) across the four sites varies from season to season attaining highest value in rainy season and low during winter season thereby indicating high immobilization of microbial C and N during the rainy season. The present value of microbial C to organic C falls within the reported range of tropical forests (1.5 – 5.3 %; Theng et al., 1989; Luizzao et al., 1992) and temperate forests (1.8–2.9 %; Vance et al., 1987). However, the contribution of microbial biomass N to total N is comparable the values reported from agricultural soils (2–6%; Brookes et al., 1985), acid organic soils (2.8–9.8%; Williams and Sparling, 1984) and forest soils (3.4– 5.9 %; Martikainen and Palojarvi, 1990). Contribution of microbial P to total Phosphorus (2.46 – 5.20 %) across the sites indicates higher immobilization and is comparable to the values reported by Brookes et al. (1984) from deciduous woodland (4.7 %), grasslands (2–4.3 %) and arable land (1.4–3.5 %) and it falls well within the reported range of Yadava and Devi (2004) from semi evergreen forest (2.74 %) but lower than that of sub-tropical humid forest of North-east India reported by Arunachalam and Arunachalam (2000) owing to high microbial P and low pH in those forests.

Conclusion

Thus, it may be concluded that the soil microbial biomass exhibits strong seasonality and is highly influenced by the abiotic variables. However, soil moisture has a strong influence on the microbial biomass. The microbial C:N ratio indicates that soil fertility is influenced by the species composition of the forest. High microbial N during the rainy season may be onsidered as a nutrient conservation strategy. Further, the proportion of microbial C and N to soil C and N indicates that C, N and P are immobilized more during rainy season. Results from the present study demonstrate that management practices and certain types of vegetation exert a profound influence on microbial biomass C and N. Different plant species affect soil microbial processes, which are dependent upon their litter quality and quantity and also upon below-ground biomass supporting microbial activities. The substrate and nutrient limitation of microbial biomass and their central role in the soil nutrient cycling facilitate the use of microbial biomass as an indicator for soil health of land use types. Our data suggest that forest soil may be healthier when compared to other land use soils. In other words, the soil health of land use types is in the order of forest, plantation, grassland and agriculture soil.

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