Chapter 2

General overview of materials and methods Used in the field trials

This chapter describes the general materials and methods used in the field experiments discussed in Chapters 4-7. The materials and methods specific to individual experiments are covered in their respective chapters. Two of the field studies were carried out in 7 and 10 years old trials.

2.1 Site description

My field studies were conducted in existing long-term field trials at Makoka Agricultural Research Station near Zomba in Southern Malawi (15^{o} 30' S, 35^{o} 15' E, altitude of 1029 m asl). The Research Station is in Thondwe Highlands ecological zone. The soils of Makoka Agricultural Research Station closely represent the common soils in the Thondwe Highlands ecozone. Gneisses and granulites of the Basement Complex underlie the Thondwe highlands. The plains have moderately to deep soils, which are well drained and have medium to fine texture, and moderate chemical fertility (Venema, 1991). The soils at the three trial sites are classified as Ferric Lixisols (FAO) (Ikerra *et al*., 1999, 2001). The three fields are coded MZ12, MZ18 and MZ21. Soil characteristics of the experimental fields are presented in Table 2.1.

Table 2.1. Summary of the characteristics of topsoil (0-20 cm) at MZ12, MZ18 and MZ21 assessed at the time of establishments of experiments at Makoka Agricultural Research Station, Zomba.

Soil parameter	MZ12	MZ18	MZ21
Physical characteristics			
Clay (%)	42	33	38
Sand (%)	46	57	54
Silt (%)	12	10	8
Bulk density (g/cm^3)	1.42	1.27	1.55
Chemical characteristics			
pH in water (1:2.5)	5.9	5.8	5.6
Organic carbon (g/kg of soil)	8.8	4.2	9.3
P-Olsen (mg/kg of soil)	26.0	12.5	10.3
Exchangeable K (mmol (+)/kg of soil)	3.0	2.6	3.7
Exchangeable Ca (mmol (+)/kg of soil)	44.0	47.7	17.3
Exchangeable Mg (mmol (+)/kg of soil)	16.0	13.7	4.2

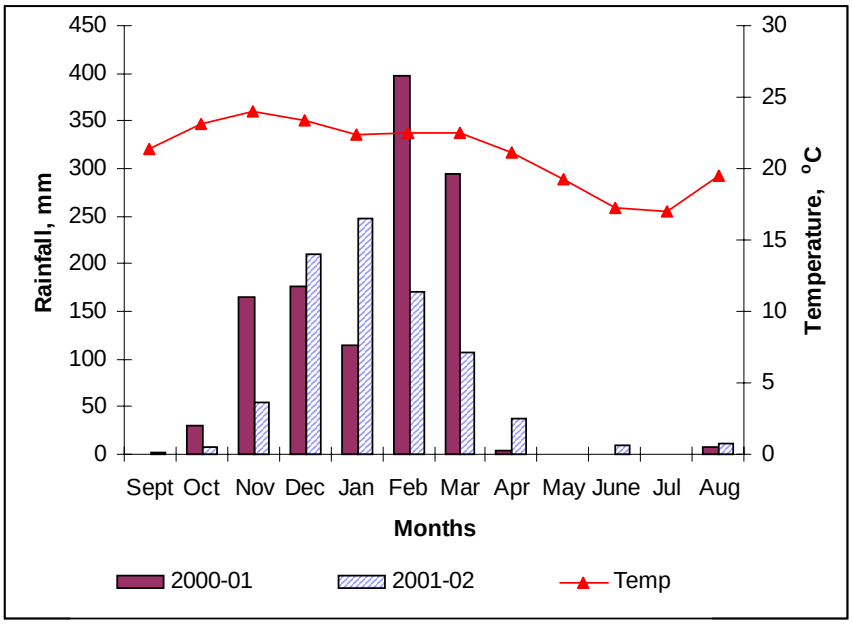

Fig 2.1. Monthly rainfall means for 2000-01 and 2001-02 and mean monthly temperature averaged over the two seasons. (Data obtained from the meteorological station at Makoka Agricultural Research Station)

The rain season starts in November and ends in March with most of the rain falling between December and January. Usually the rainfall is very sporadic during the first month of the rainy season. The annual rainfall averaged over the past 10 years was 930 mm. The onset dates and distribution of the rainfall are unpredictable and unreliable. Figure 2.1 presents the monthly mean rainfall for the two seasons of experimentation. The rainfall graphs showing the daily rainfall trends during the maize growing period have been presented in Chapters 4 and 5. In 2000-01 the rain started in October with sporadic rainfall, but intensified in February (396 mm) and March (295 mm), whereas in 2001-02 the rainfall commenced in November and peaked in January (248 mm) and tailed off in March (26 mm).

2.2 Tree and crop management

The studies were conducted in fields with the following agroforestry technologies:

(1) Gliricidia-maize simultaneous intercropping, at MZ12 and MZ21

(2) Sesbania-maize relay intercropping, at MZ21.

(3) Gliricidia-maize/pigeonpea and sesbania-maize/pigeonpea intercropping, at MZ21

(4) Gliricidia biomass transfer at MZ 18

In addition we had sole maize cropping system as a control in all the fields and maize-pigeonpea intercropping system at MZ21 field.

2.2.1 Gliricidia-maize simultaneous intercropping

Two long-term trials with gliricidia intercropping were used; the first trial was established in December 1992 and is coded MZ12 and the second trial was established in December 1995 and is coded MZ21. In both trials *Gliricidia sepium* (Jacq.) Walp ex Reltahaleu, Guatemala was used. The gliricidia trees were planted in rows in every other furrow at spacing of 90 cm within row and 150 cm between rows (7400 trees ha^{-1}). Figures 2.2a and 2.2b present the spatial arrangement of trees and maize. About 2 weeks prior to planting maize, gliricidia was pruned to about 30 cm height, and the prunings were arranged in the opened ridges and covered with soil at a depth of 15 cm during land preparation (Appendix 2.1a). Gliricidia biomass was cut three times per season, in October, December and February. A pre-cut in July was necessary to remove the old woody biomass and encourage new growth for the cut of October. The leaves from the pre-cut were also incorporated in the soil at MZ12. In the experiment at MZ21, where the amount of gliricidia prunings was fixed at 3 tons ha^{-1} and all applied at once, only the prunings from the cut of October were incorporated. At MZ12 where the quantity of gliricidia prunings was not fixed, all the organic materials from each cutting were incorporated. MZ21 was the main field for most of the experiments reported in this thesis, whereas at MZ12 root studies were done and soil nutrient parameters were assessed after 10 years of continuous cropping. (The experiment layout, plot sizes and treatment combinations for each site are given in the specific chapters wherein reported).

2.2.2 Sesbania-maize relay intercropping

In the trial with sesbania relay intercropping, the maize was planted first, at the rain onset, and nursery raised sesbania seedlings were planted 2 weeks after planting maize. In this experiment the species of *Sesbania sesban* (L.) from Zalewa Malawi was used. Sesbania trees were planted along with the maize in every other furrow at

spacing of 90 cm within row and 150 cm between rows (7400 trees ha^{-1}) similar to gliricidia (Fig. 2.2b). In October, at the time of land preparation the trees were felled and the prunings (litter, fresh leaves and twigs) were arranged in the furrows, which were then covered with soil from the old ridges constituting a new ridge over the biomass (Appendix 2.1b). Sesbania trees were replanted every season two weeks after maize planting. The sesbania plots have been maintained since 1995.

2.2.3 Pigeonpea intercropping

Pigeonpea was planted at the same time as maize. Pigeonpea was planted as an intercrop. Pigeonpea was intercropped with maize, and in maize with gliricidia and sesbania treatments. Four seeds of pigeonpea were planted per hole on the ridge in the space between maize plants. Two weeks after germination the seedlings were thinned to three. Long maturing, local variety was used because firstly its initial slow growth reduced competition for the above ground resources with maize and secondly it has high biomass production capacity. After harvesting the maize in May the pigeon pea took over and was allowed to grow even after the peas had been harvested in August. The biomass production varied between 0.8 t/ha (intercropping with gliricidia and sesbania) and 2 t/ha (intercropped with maize only). The pigeonpea was cut at the time of ridge making. Pigeonpea fresh leaves, twigs and leafy litter were combined and will be referred to as pigeonpea leaves.

2.2.4. Gliricidia biomass transfer

MZ18 trial had 28 plots per replicate, 4 of which had gliricidia trees. The trial plots with gliricidia trees were not cropped but used for production of biomass. The biomass was cut from the plots with trees and was transferred to incorporate in plots without trees. Since we could not get enough gliricidia biomass from the 12 tree plots within the field to apply in the treatments, we had to carry some gliricidia biomass from a different field with trees of the same age. Gliricidia prunings were applied on 23rd October, 22nd December 2001 and 22nd February 2002. In the preceding season (2000-01) maize was planted without inorganic or organic fertilizer amendment in order to deplete/lower the nutrients in the soil. Trenches, 50 cm wide and 100 cm deep, were dug between the plots with and without trees, and all the roots extending into the neighboring plots without trees were cut.

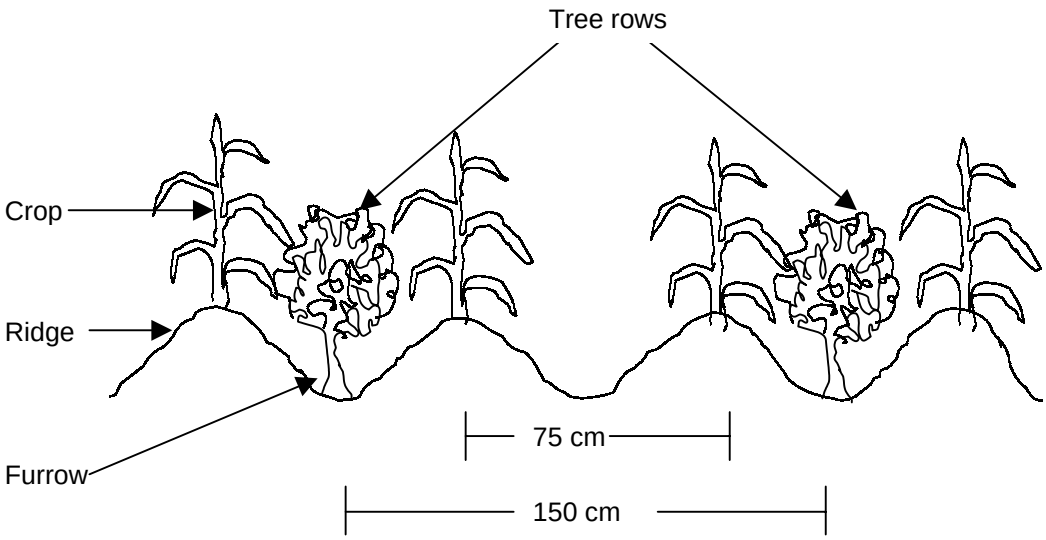

Fig. 2.2a. Cross sectional view of the spatial arrangement of gliricidia rows and maize

Fig. 2.2b. Spatial arrangement of trees and crops in the gliricidia simultaneous intercropping with maize agroforestry system

2.2.5 Maize cropping

At rain onset maize hybrid NSCM 41 was planted on the ridges at a rate of 3 seeds per hole 90 cm apart within ridge and 75 cm between ridges (44 400 plants ha^{-1}). At MZ12 the maize was planted at a rate of 1 seed per hole at 30 cm apart maintaining

the same population. Weeding was done twice by hand. In both seasons the maize was harvested in May. Maize stover, rachis and grain samples were collected at harvesting and were dried in an oven at 75 ^{o}C for 48 hours, and dry-mater (DM) content was determined. The DM content of maize grain was used to convert grain yield into yield at 13% moisture; hence the maize grain yield herein reported refers to grain with 13% moisture content.

2.3 History of the field trials

2.3.1 Experimental field at MZ12

Since the establishment of the agroforestry experiment in 1992 the plots have been continuously cropped. The experiment at MZ12 has the following treatments: Sole maize, gliricidia intercropped with maize with and without inorganic N (24 and 48 kg/ha) and P (20 and 40 kg/ha). The biophysical data for this long term experiment has been reported by Akinnifesi *et al.* (in press). For the root studies reported in Chapter 6 and carbon sequestration in Chapter 7 data was collected from the treatments without inorganic fertilizers.

2.3.2 Experimental field at MZ21

The agroforestry experiment established in 1995 had the following cropping systems: sole maize, gliricidia-maize simultaneous intercropping and sesbania relay cropping and treatments imposed in these systems are shown in Table 2.2. The maize yield and pigeon pea and tree biomass yields are reported by Makumba *et al.* (2000). The treatments were slightly changed in 2000/2001 to new treatments for the studies reported in Chapter 5. The new treatments adopted the plots of the old treatments as shown in Table 2.2.

In the 1999/2000 season prior to the establishment of the new experiments discussed in Chapter 5 the field was cropped without N fertilizer. The trees were pruned as usual but the biomass was applied elsewhere. The 1999/2000 season was used to remove any residual effect of the low doses of N fertilizer applied in the previous experiments. Results of maize yields in the 1999/2000 indicate that the residual effect was negligibly small (yield differences between control and N fertilizer treatments were <100 kg maize per ha) (Makumba *et al*., 2000).

Table 2.2. New treatment allocation in the old treatment plots at MZ12 as of October 2000.

Old	New[3]
Sole maize	Sole-Maize (NTP/NCR)
Maize + 24 kg N/ha (F1)[1]	Maize (1.5 t/ha maize stover incorporated)
Maize + 24 kg N ha split (F2)[2]	Maize (3 t/ha maize stover incorporated)
Maize/pigeonpea intercropping	Maize/pigeonpea (pigeonpea leaves applied)
Maize/pigeonpea intercrop.+F1	Maize/pigeonpea (pigeonpea roots applied)
Maize/pigeonpea intercropping + F2	Maize/pigeonpea (pigeonpea leaves & roots)
Gliricidia-Maize	Gliricidia-maize
Gliricidia-Maize + F1	Gliricidia-Maize (1.5 kg/ha maize stover)
Gliricidia-Maize + F2	Gliricidia-Maize (3 t/ha maize stover)
Gliricidia-Maize/pigeonpea	Gliricidia-Maize/pigeonpea (pigeonpea leaves)
Gliricidia-Maize/pigeonpea + F1	Gliricidia-Maize/pigeonpea (pigeonpea roots)
Gliricidia-Maize/pigeonpea + F2	Gliricidia-Maize/pigeonpea (pigeonpea leaves & roots)
Sesbania-Maize	Sesbania-maize
Sesbania- Maize + F1	Sesbania-Maize (1.5 kg/ha maize stover)
Sesbania-Maize + F2	Sesbania-Maize (3 t/ha maize stover)
Sesbania- Maize/pigeonpea	Sesbania-Maize/pigeonpea (pigeonpea leaves)
Sesbania- Maize/pigeonpea + F1	Sesbania-Maize/pigeonpea (pigeonpea roots)
Sesbania- Maize/pigeonpea + F2	Sesbania-Maize/pigeonpea (pigeonpea leaves & roots)

[1]F1 = 24 kg N/ha, all applied at once at 4 weeks after planting

[2]F2 = 24 kg N/ha, applied as split 12 kg N/ha at planting and 12 kg N/ha 4 weeks after planting. [3]Tree prunings were applied in their respective plots in quantities as described in Chapter 5.

2.3.3 Experimental field at MZ18

Originally, MZ18 was designed as a screening trial of different agroforestry shrubs in an improved fallow. The fallows were cleared in 1997 and the plots were cropped with maize during 1997/1998 and 1998/1999 seasons without addition of any inputs. In 2000 the site was again cropped with maize without applying any soil amendments to exhaust any residual N in the soil prior to establishment of the time of application trial reported in Chapter 4.

2.4 Soil sampling and analyses

Soil samples were collected from the experimental plots at the time of land preparation in October and later during the season. The initial soil samples collected during land preparation were sampled from the plots from the 20 cm soil layer. Ten locations were augured per plot and were bulked. The bulked soil samples were subdivided into two; one sub-sample was stored in a fridge at 4 ^{o}C pending mineral N analysis, and the other sub-sample was air dried and sieved through 2 mm mesh and analyzed for organic carbon (OC), pH, available P, K, Ca, Mg and texture.

During the rain season soil samples were collected from 0-20 cm soil layer, once a month from November to March. In December and February soil samples were collected up to 200 cm at 20 cm depth intervals. The soil samples were collected using Edelman's auger. Five points were augured within the net plot and samples collected from the five locations at the same depth were mixed thoroughly and a bulk sample was placed in a well labeled plastic bag. These samples were extracted by 2 M KCl and analyzed for mineral N (NH_4-N + NO_3-N) only. Total organic carbon in soil was analyzed following the "wet" oxidation by acidified dichromate method according to Anderson and Ingram (1993). Soil samples were sieved through a 0.1 mm mesh. 1 g sub-samples were weighed into 75 ml digestion tubes to which 10 ml of 5% potassium dichromate and 5 ml concentrated (98%) sulfuric acid were added. The tubes were digested at 150 ^{o}C for 30 minutes, and the solutions were left overnight to settle. Total organic carbon was determined in the clear supernatant at 600 nm on a colorimetric spectrometer.

Mineral N was determined from fresh soil samples. About 20 g field moist soil was extracted with 100 ml of 2 M KCl. The samples were shaken on a horizontal shaker at 250 oscillations per minute for one hour, filtered through a pre-washed Whatman No. 5 filter paper. A second sub-sample of soil was dried at 105 ^{o}C for 24 hrs to determine the dry weight of extracted soil. Ammonium-N was determined in the extracts by colorimetric method by Anderson and Ingram (1993). Nitrate-N was determined by cadmium reduction (Dorich and Nelson, 1984) and subsequent colorimetric analysis of nitrite. In the subsequent chapters the sum of the NH_4-N and NO_3-N is referred to as total mineral N.

For determination of soil reaction, pH (H_2O), 25 ml of distilled water was added to 10 g of soil and the solution was shaken on a horizontal shaker for 2 hrs. The suspension was left to settle and pH was measured in the supernatant (Houba *et al*., 1979).

Available P was determined in the soil according to the method of Watanabe and Olsen (1965). To 2.5 g of soil, 50 ml of 0.5 M $NaHCO_3$ solution buffered at pH 8.5 was added and the samples were shaken for 30 min., and then filtered through Whatman no. 42. In the filtrates P was determined according to a molybdate-ascorbic acid method.

Exchangeable cations, K, Ca and Mg were extracted from the soil using 1 M ammonium acetate buffered at pH 7. In the extract K was determined on a flame photometer. Ca and Mg were determined on atomic absorption spectrophotometer (AAS).

2.5 Plant sampling and analyses

2.5.1 Sampling

Gliricidia and sesbania foliar samples were collected twice. The first sampling was done one week prior to pruning. The foliar samples were randomly collected from five trees from the net plot. Pre-weighed foliar samples were dried at 75 ^oC to constant weight and their dry-matter contents were determined. The dry-matter contents of the organic material were used to convert the fresh weights of the prunings into equivalent dry weights at the time of cutting. The samples that were analyzed for N, P and C contents were sampled at the time of incorporation.

Plant samples collected from belowground parts of the plant were first washed to remove the soil, rinsed in distilled water, and dried in an oven at 75 ^oC for 48 hrs. Large roots and tree stems were sliced into thinner pieces to allow for fast drying and the drying period was extended until the roots reached constant weight. The dried plant materials were finely ground and stored in tightly closed bottles.

2.5.2 Digestion and analyses

To 0.3 g of the finely ground materials, 2.5 ml of H_2SO_4-Se solution was added. The mixture was left to stand overnight and then heated at 100 ^oC for 2 hrs. 3 ml of hydrogen peroxide was added and the samples were further heated at 330 ^oC until the solution turned colorless. In the solution total N was determined by colorimetric method, P was determined by a molybdate-ascorbic acid method, and K with a flame photometer (Temminghoff *et al*., 2000).

2.6 Root studies

Root studies were undertaken following the procedures described by Akinnifesi *et al*. (1999) and Vanlauwe *et al.* (2002). Trenches, 250 cm long, 100 cm wide and 300 cm deep, were dug eight weeks after planting maize. At MZ12 the trenches were dug perpendicular to the ridges and tree rows (see Fig 2.3) at 5 cm away from the maize plant and 15 cm away from the trees.

A t MZ21 the trenches were dug parallel to the tree rows at 5 cm away from the maize and pigeon pea row and 42 cm from the tree row (see Fig. 2.4). The pits were dug parallel to the tree rows at MZ21 because we wanted to expose both the pigeon pea and maize roots. Root samples collected earlier on the ridge where maize and pigeon pea were growing had shown that pigeon pea roots hardly grew beyond the maize planting holes and vice versa; they did not extend beyond 45 cm. As pigeon pea was planted in between the maize planting holes on the same ridge, perpendicular positioning of the trench could mean that the pigeon pea was behind the maize plant and that the pigeon pea roots could not be exposed on the profile wall. We were also interested in studying the overlapping of pigeon pea and maize roots along the ridge where most of their roots were growing and feeding from.

2.7. Data analysis

The primary data was subjected to ANOVA using GENSTAT version 5. Significant test for the mean separation was done by Duncan Multiple Range Test (DMRT). Simple correlation coefficients were determined for the linear relationships between variables. The root length density and soil mineral N data were log10 (n+1) transformed before subjecting to ANOVA (Gomez and Gomez, 1984).

Fig 2.3. Mapping of roots at MZ12 in a sole maize plot, trenches were dug perpendicular to the ridges (photo taken at the time of root mapping).

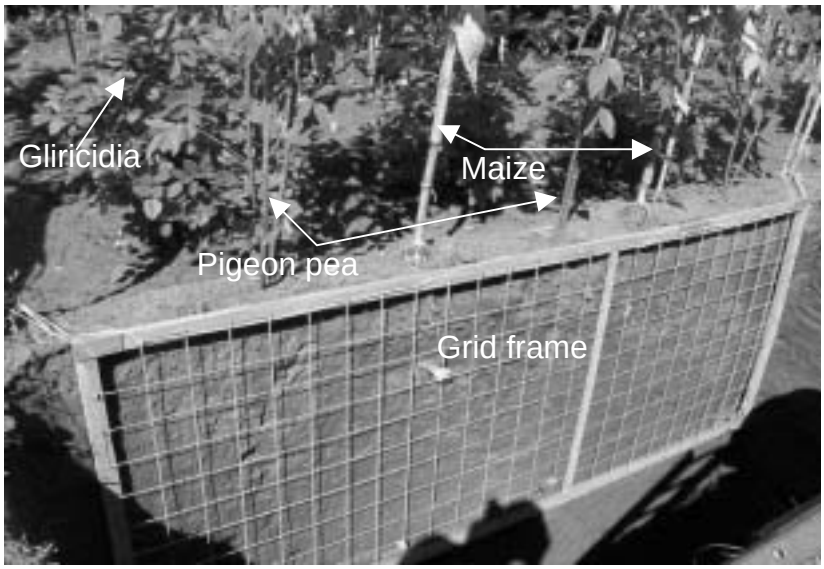

Fig. 2.4. Mapping of roots at MZ21 in a Gliricidia-maize-pigeon pea intercropping system. Trenches were dug parallel to the ridges and tree rows.

Appendix 2.1a. Arranging gliricidia prunings on split ridges

Appendix 2.1b. Arranging sesbania prunings in the furrows